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Review

# An update on the cardiac effects of erythropoietin cardioprotection by erythropoietin and the lessons learnt from studies in neuroprotection

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**Abstract**

Erythropoietin (Epo) was once thought to act exclusively in the formation of red blood cells. As recently reviewed by Smith et al. [Cardiovasc. Res. 59 (2003) 538–548], Epo can also act within the cardiovascular system with effects in thrombosis and hypertension as well as actions on platelets, vascular endothelium and smooth muscle, and myocytes of the heart. Here, the actions of Epo to protect neuronal cells of the brain are first evaluated and parallel actions of Epo in cardioprotection are then drawn. Thus, with recent reports of Epo receptor (EpoR) expression by cardiac myocytes, it could be predicted that Epo initiates direct protective signalling events. This is supported by five independent studies published in 2003 showing Epo protects cardiac myocytes following ischemia/reperfusion. Importantly, these protective actions have been observed in vitro and in vivo. The former suggests the direct actions of Epo to prevent myocyte death independently of its effects on red blood cell number or cells other than cardiac myocytes. The latter demonstrates the potential for Epo in the treatment of the heart post-infarction, decreasing the numbers of apoptotic myocytes, limiting infarct expansion and attenuating the post-infarct deterioration in haemodynamic function. These beneficial effects of Epo should stimulate further research into the actions of Epo.


*Keywords:* Apoptosis; Phosphatidyl inositol 3′ kinase; In vitro cardioprotection; In vivo cardioprotection; Ischemia/reperfusion damage

## 1. Introduction

Smith et al. [1] recently presented an overview of the cardiovascular effects of Erythropoietin (Epo). Importantly, Epo was once thought to act exclusively to direct the formation of erythrocytes. Thus, Epo in its recombinant form is used widely in the treatment of anaemia associated with renal failure, Human Immunodeficiency Virus infection, cancer and surgery [2–5]. As highlighted [1], Epo has broader actions independent of its effects on erythrocyte numbers. In particular, many studies have now shown neuroprotection by Epo, apparently through its direct actions on cells of the brain. Here, we outline these studies on neuroprotection and emphasise the intracellular signalling pathways that may mediate these actions. We then go on to describe five studies published in 2003 showing Epo is cardioprotective [6–10]. These independently show that Epo reduces injury of the heart following ischemia and reperfusion. Thus, the use of Epo may become an increasingly attractive option for patients with heart failure and anaemia [11], as well as for those with acute cardiac damage following myocardial infarction. In the following section, we focus on the direct protective actions of Epo, beginning with the evidence supporting a role for Epo in neuroprotection.

## 2. Actions of Epo in the brain—a paradigm for cellular protection by Epo?

In response to low oxygen or ischemic events, Hypoxia-Inducible Factor (HIF) regulates the expression of a number of critical hypoxia-inducible genes including that for Epo (Fig. 1) [12]. An initial surprise was that the adult kidney

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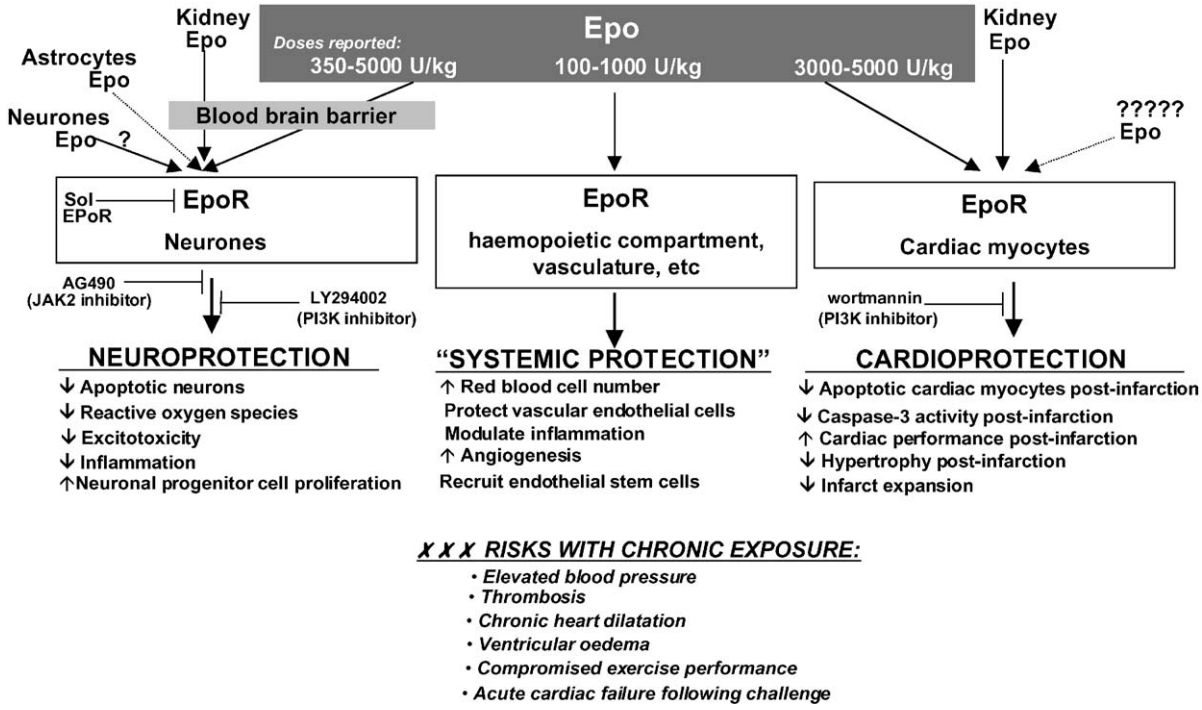


Fig. 1. Schematic diagram of the possible actions of Epo in neuroprotection, "systemic protection" and cardioprotection. The predominant natural source of Epo is the kidney following hypoxia, however, there is evidence that astrocytes and neurons of the brain can also produce Epo under conditions of low oxygen. As yet, there are no reports of endogenous cardiac production of Epo. Exogenous Epo is now being tested for its roles in neuroprotection, "systemic protection" and cardioprotection, and its protective actions are listed. Importantly, high doses of Epo might need to be administered to achieve neuroprotection, as the delivered Epo must cross the blood–brain barrier before it is able to interact with the receptor for Epo, EpoR, on the neurons. In contrast, similar barriers do not exist for other cells of the body, including the cells of the heart and vasculature. There are also a number of risks that have been reported either in patients with chronic Epo administration, or following chronic upregulation of systemic Epo levels in transgenic animals. The benefits and risks will need to be evaluated more carefully, but acute Epo administration appears as an attractive new treatment strategy for stroke and cardiac infarction.

was not the sole producer of Epo. Specifically, low oxygen tension enhances Epo mRNA expression in the astrocytes and neurons of the brain [13,14], so that these cells become sources of Epo. Because the Epo receptor (EpoR) is expressed by these astrocytes and neurons [15,16], this potentially allows paracrine and/or autocrine signalling to the neurons in the brain during times of hypoxia and/or ischemia. In vitro studies using the transfer of conditioned media suggest that paracrine rather than autocrine signalling protects neurons during oxygen–glucose deprivation [17], although autocrine signalling cannot be dismissed under other in vitro or in vivo conditions. More recently, in vivo studies have demonstrated that the protection of hypoxic preconditioning in the brain could be partially attributed to enhanced local Epo production because the administration of a soluble form of the EpoR could block protection [18]. Similarly, ischemic death of neurons was exacerbated in the presence of soluble EpoR [19]. These studies highlight the importance of endogenous Epo. They also suggest that Epo supplementation may be useful in the treatment of stroke.

In neuronal injury models, the administration of recombinant Epo protects against ischemia and free radical injury. Of the many examples of this successful approach, Epo (5000 units/kg, intraperitoneal injection) when administered at the time of the reversible occlusion of the middle cerebral artery, or 6 h later, decreased the volume of cerebral infarction by as much as 75% [20,21]. Outside the brain, Epo delivered intravenously (350–1000 units/kg) immediately after reperfusion prevented motor neuron apoptosis when the spinal cord was injured by ischemia following a 20-min occlusion of the abdominal aorta [22]. In addition to decreased cell death, there was less neurologic disability in the Epo-treated animals [22]. This is an important endpoint, showing that Epo delivery following an insult can preserve complex physiological functions. Interestingly, the doses of Epo used in these studies on neuroprotection in the brain were higher than those routinely used (100–1000 units/kg) in the treatment of anaemia [23–25]. Although Epo is a 30.4-kDa glycoprotein, it has been shown to cross the blood–brain barrier [20]. However, this remains controversial with others reporting that the blood–brain barrier is either not permeable, or may only be permeable to Epo following an insult such as stroke [26,27]. It may be that the direct actions of Epo on neurons are only possible when sufficiently high levels of Epo are achieved following high dose administration, so that its transfer across the blood–brain barrier is possible. Support for this contention comes with the clinical trial of Epo in the treatment of stroke [28].

Together with a strong trend for reduced infarct size and improved follow-up and outcome scales, Epo in the cerebrospinal fluid of stroke patients increased 60–100 times following intravenous Epo administration at a dose of 33,000 units per day for 3 days [28].

Studies confirming the direct actions of Epo have exposed in vitro cultures of neurons to recombinant Epo. For example, cultured neurons were protected from glutamate-induced or nitric oxide-mediated neurotoxicity if pretreated with Epo for 8 or 24 h, respectively [19,29]. Other in vivo studies have also dissociated the direct protective effects of Epo from its ability to alter erythrocyte numbers. For example, the in vivo administration of an asialoform form of Epo with a shortened half-life did not change blood haemoglobin levels and remained neuroprotective [30]. These results open new avenues for the use of asialo-Epo because neuroprotection appears possible without increased erythrocyte numbers that amplify brain injury [31]. Asialo-Epo may also avoid the complications of hyper-reactive platelets and alterations in the endothelium that predispose patients to thrombosis [32,33]. Therefore, asialo-Epo is showing excellent therapeutic promise.

The actions of Epo in neuroprotection, independent of changes in erythrocyte numbers, can now be broadly divided into two categories. First, Epo can act within the in vivo context to reverse vasospasm [34,35], protect vascular endothelial cells [36], modulate inflammation [20,37,38] and recruit stem cells [39]. Second, Epo can act directly on neurons. Thus, Epo can attenuate the production of damaging molecules such as reactive oxygen species or glutamate-stimulated excitotoxicity [16,40,41]. This likely contributes to lower levels of apoptosis [16,21,22]. Therefore, Epo can act at multiple levels to protect neurons.

The evaluation of signal transduction pathways involved in neuroprotection has predominantly focused on the direct actions of Epo on neurons. The use of the inhibitor AG-490 has implicated JAK2 of the Janus Kinase (JAK) family of tyrosine kinases [17]. Thus, when JAK2 was inhibited, phosphorylation of the inhibitor of NF-κB and the subsequent nuclear translocation of NF-κB were prevented and NF-κB-dependent transcriptional changes to increase neuroprotective proteins [16] would be inhibited. Perhaps surprisingly, the Signal Transducer and Activator of Transcription (STAT) family of transcription factors such as STATs 1, 3 or 5, which act immediately downstream of JAKs and which contribute to antiapoptotic signalling in non-neuronal cells were not activated in Epo exposed neurons [17]. Nor could STAT-transactivated anti-apoptotic genes be detected [17].

The mitogen-activated protein kinases (MAPKs) have been implicated in cellular protection in a variety of cell types. But again, the activation of the ERK or p38 subfamilies of MAPKs was not observed in Epo-treated neurons [17]. In contrast, others have noted acute ERK activation in Epo-treated neurons [21]. Despite these inconsistencies, it could be argued that the most appropriate experiments should evaluate the signalling pathways activated by Epo during hypoxia or ischemia/reperfusion when conditions more closely resemble the infarcted brain. Thus, Epo treatment of hippocampal neurons under hypoxic conditions appeared to maintain phosphorylated STAT5 and ERK MAPKs at levels above the hypoxic, non-Epo-treated cells [21]. It may be that maintenance of these protective pathways, rather than acute activation by Epo in the normoxic state, is a greater contributor to signalling neuroprotection.

There appears to be more substantial evidence that the lipid kinase Phosphatidylinositol 3′ Kinase (PI3K) is involved in Epo-dependent cell survival with the demonstration that the PI3K inhibitor LY294002 inhibits Epo-driven neuroprotection [17]. Epo activates EpoR so that the dual SH2-domains of the p85 regulatory subunit recruit PI3K to specific phosphotyrosine residues within the cytoplasmic domain of EpoR [42] or to the adaptor-like protein insulin-receptor-substrate-1 [43]. This places PI3K in close proximity to its substrates where it phosphorylates membrane phosphoinositide lipids [44]. The protective actions of PI3K are most likely mediated via the downstream Akt family of serine/threonine protein kinases [45]. Akt phosphorylates its substrates at the consensus motif RXRZZ-S/T-Hyd (where X is any amino acid, Z represents a small residue other than Glycine and Hyd is a bulky hydrophobic residue such as phenylalanine or leucine) [46]. Although database searching shows many proteins with this general sequence ( ~ 14,000 sites identified in ~ 9500 proteins [47]), a smaller number of these are experimentally confirmed as Akt substrates in vitro and in vivo. For example, Akt can phosphorylate and inhibit pro-apoptotic proteins such as Bad [48]. Protective actions appear dependent on the preservation of mitochondrial membrane integrity thus preventing cytochrome *c* release and subsequent activation of the caspase family of pro-apoptotic proteases [49].

There are also less direct actions of Akt that could contribute to its potent pro-survival/anti-apoptotic signalling. For example, Akt can phosphorylate Mdm2 to enhance its ubiquitin ligase activity and hence degradation of p53 [50]. This suppresses the actions of the transcription factor p53 [51] and therefore inhibits p53-driven expression of pro-apoptotic proteins such as the Bcl2 family members Noxa [52] and Bax [53]. Akt activation also contributes to the activation of NF-κB, which can enhance production of anti-apoptotic proteins such as BcL-xL and A1/Blf-1 [54,55] and the inhibitor of apoptosis, IAP [56]. Thus, multiple signalling events downstream of Akt contribute to neuroprotection by Epo by enhancing anti-apoptotic events and suppressing pro-apoptoptic events.

These studies raise the questions of whether other non-neuronal cells can also be protected by Epo and which anti-apoptotic pathways would be employed in these other cells. In the following sections, we turn our attention to the recently described cardioprotective effects of Epo.

## 3. Long-term and indirect effects of Epo contributing to cardioprotection

The ischemic injury of the brain following stroke or the injury of the heart following infarction can be characterised by a central region of rapid and predominantly necrotic cell death. This is surrounded by the volume at risk in which cells undergo slower apoptotic cell death [57]. Treatments to preserve the myocardium following infarction, like those that preserve the brain following stroke, aim to eliminate or lessen the ischemic episode. This is illustrated by the use of angioplasty, thrombolytics or platelet antagonists (as reviewed by Theroux et al. [58]). Whilst these are proving useful, their immediate actions to reestablish blood flow are predominantly reactionary without protecting against future ischemic episodes.

Linked with these longer-term approaches to enhance or reestablish blood flow are the beneficial effects of Epo to enhance oxygen delivery. Thus, Epo, as produced by the kidney in response to hypoxia or administered therapeutically, can stimulate erythroid progenitors to increase the number of mature red blood cells and increase the oxygen carrying capacity of the blood [5]. Single dose Epo can increase erythroid progenitor numbers in the form of reticulocytes in 3–4 days with maximal levels at 8–11 days [59]. Whilst subsequent increased oxygen carrying capacity may help, this unlikely to contribute to protection in acute (<24 h) time points before reticulocyte numbers increase.

In the context of Epo-dependent cardioprotection when Epo is given therapeutically, it is of interest that exogenous Epo protects vascular endothelial cells from apoptosis [36], mobilizes endothelial progenitor cells [60,61] and acts as an angiogenic factor [62]. Indeed, Epo induces both early angiogenic events such as increased cell proliferation and matrix metalloproteinase-2 production, and late angiogenic events such as differentiation into vascular tubes [63]. When compared with Vascular Endothelial Growth Factor, Epo exerted equivalent capillary outgrowth of endothelial cells derived from adult human myocardial tissue [64]. These actions would contribute to improved cardiac recovery through both maintaining and re-establishing vasculature.

However, Epo administration is not without its problems, especially when chronically administered. Epo can elevate blood pressure and the incidence of thrombosis [65]. Increases in hematocrit have been linked with excess mortality rates in patients with ischemic heart disease [66,67]. Such problems are also observed in transgenic animals overexpressing Epo. Specifically, larger infarct zones with enhanced leukocyte infiltration were observed in transgenic mice with increased systemic Epo levels and elevated hematocrits [31]. These Epo-overexpressing animals also had increased heart weight with ventricular dilatation and intracellular oedema [68]. Exercise performance was compromised, and life expectancy was significantly shortened [68]. In response to norepinephrine infusion, a majority of Epo-overexpressers died or required resuscitation due to severe diastolic dysfunction, myocardial ischemia and ultimately acute heart failure [69].

The exact mechanisms underlying these pathological changes accompanying systemic Epo elevation remains to be fully defined, however recent data shows that endothelin levels are increased in the aorta, liver, heart and kidney of mice overexpressing Epo [70]. Use of an endothelin-receptor-A antagonist has led to the suggestion that endothelin expression contributes to the cardiovascular pathologies accompanying chronic Epo overexpression [70]. These broader effects of Epo must be carefully considered, especially if Epo is to be considered for long-term treatment.

A second treatment strategy aims to preserve the myocardium following trauma. In contrast to those approaches described above, this is aimed directly at protecting heart cells from ischemia or hypoxia-induced cell death. In this way, these treatments help maintain viable myocardium. This can be achieved through the use of suitable cardioprotective agents of which antioxidants, calcium channel blockers, nitric oxide, adenosine-related agents, inhibitors of the renin–angiotensin system, endothelin receptor antagonists and sodium–hydrogen exchange inhibitors are currently amongst the best characterised [71]. An ideal strategy here would be one in which treatment is initiated as soon as possible after the ischemic episode. An effective cardioprotective treatment would allow enhanced cell survival despite the hostile environment of the infarct. In the following sections, we describe these direct actions of Epo to protect the cardiac myocyte.

## 4. In vitro assessment of the cardioprotective properties of Epo

For Epo to exert direct actions on cardiac myocytes, these cells must express the appropriate receptor. EpoR can be found in the heart as shown in a recent study using Reverse Transcriptase-Polymerase Chain Reaction of adult mouse heart [7]. The potential disadvantage of this approach is that it this does not exclude expression on non-myocytic cells of the heart, and that the levels of expressed protein have not been detected. These issues are addressed by immunohistochemical staining and immunoblotting of neonatal rat ventricular cardiac myocytes, which has shown that these cells do express EpoR [9]. Epo itself has not been detected in the heart either under normoxic or mild hypoxic conditions [72]. However, HIF levels do increase in hypoxic myocytes [73], suggesting that HIF-dependent changes such as enhanced Epo expression are possible. The transcription factor GATA4 also regulates Epo expression [74] and contributes to gene expression changes in the heart [75], so it remains to be more closely evaluated whether cardiac myocytes express Epo to allow autocrine signalling in the heart.

In the simplest experimental system showing cardioprotection by Epo, cardiac myocytes have been exposed to Epo and subjected to in vitro conditions that mimic the stresses experienced either during or following infarction. Thus, any

beneficial effects of Epo could not be an indirect result of chronic effects associated with increased erythrocyte numbers and enhanced oxygen delivery. In these protocols, the endpoint has been an estimate of the percentage of cells dying by apoptosis or necrosis with cardioprotective agents limiting death. As will be discussed in Section 5, some of these studies have also evaluated cardioprotection by Epo in vivo.

In some of the first studies on the effects of Epo on cardiac myocytes in vitro, Calvillo et al. [6] demonstrated that recombinant human Epo protected cardiac myocytes isolated from adult rats and maintained in culture. These cells were subjected to prolonged (28 h) hypoxia in which oxygen levels were decreased to less than 3% of their normoxic levels (i.e., a final concentration of 5 mm Hg). When Epo was included at a final concentration of 100 ng/ml in the culture medium from 30 min prior to the hypoxic event, there were 50% fewer apoptotic cells at 28 h but no change in the smaller fraction of necrotic cells [6].

The cardioprotective actions of Epo have also been shown when Tramontano et al. [9] exposed neonatal rat ventricular myocytes to Epo to decrease apoptosis following chronic (72 h) exposure to hypoxia (25–30 mm Hg). Similarly, Parsa et al. [8] have shown that Epo attenuated apoptotic death of the H9c2 cardiac myoblasts exposed to either oxidative stress or anoxia for 22 or 12 h, respectively. This latter study has further implicated the PI3K/Akt pathway in these protective actions through the ability of the PI3K inhibitor, wortmannin, to abolish the protective effects of Epo. This is consistent with cardioprotection following overexpression of a constitutively active Akt either in vitro [76] or in vivo [77] or the beneficial effects of viral delivery of a constitutively active form of Akt prior to doxorubicin-induced heart failure [78].

The downstream targets of the PI3K/Akt pathway in cardioprotection however remain poorly characterised. This is confounded by studies showing that some Akt substrates (e.g., Bcl2, Bad and glycogen synthase kinase-3) are either not phosphorylated following Akt activation in cardiac myocytes or that they are expressed at low levels [77,79,80]. Transcriptional effects of chronic Akt activation in the heart include enhanced expression of insulin-like growth factor binding protein-5, which could contribute to its anti-apoptotic effects, or the downregulation of peroxisome proliferator activated receptor (PPAR)-γ coactivator-1 (PGC-1) and PPAR-α that may shift myocytes to glycolytic metabolism to preserve myocardial function during ischemia [81]. Akt can also increase the expression of the Glut4 glucose transporter to enhance glucose uptake by the cardiac myocyte [77]. However, these transcriptional changes would not account for any rapid actions of Epo in cardioprotection.

There are likely to be additional Akt-dependent events either at the level of translation or post-translational modification such as the phosphorylation of the mammalian target of rapamycin (mTOR) by Akt and the subsequent phosphorylation of 4E-BP-1 and $p70^{S6K}$ [82]. This allows enhanced translation of a specific mRNA subset bound by the initiation factor eIF-4F and/or the ribosomal S6 subunit to rapidly upregulate their levels. It remains to be detailed whether levels of pro-survival proteins are rapidly influenced in this way.

Other events not requiring translation are also possible. For example, insulin acts via a PI3K/Akt-dependent mechanism to phosphorylate endothelial nitric oxide synthase to increase nitric oxide production and contribute to its protective effects [83]. Epo may use a similar Akt-dependent mechanism in the heart. Akt may also oppose pro-apoptotic events in the heart. For example, the robust activation of the stress-activated JNK MAPK pathway that follows reperfusion [84] could be a target for Epo actions via the ability of Akt to negatively regulate the upstream activator of JNK, SEK1 [85]. Akt can also phosphorylate and inhibit the proapoptotic caspase-9, although this remains controversial because this regulatory phosphorylation site is not conserved in all species [86].

It remains likely that Akt will act in concert with other protective signalling pathways stimulated either by EpoR engagement or by the stresses encountered in the ischemic/reperfused regions of the heart. However, in the following section, we move from these mechanistic considerations to review the actions of Epo in cardioprotection of the intact heart.

## 5. In vivo assessment of the cardioprotective properties of Epo

Studies in vivo on the cardioprotective actions of Epo have, to date, have subjected the hearts of adult rats and rabbits to ischemia and reperfusion. In this section, we review these studies highlighting their logical progression.

We begin with the study by Cai et al. [7]. Here, recombinant human Epo was administered (5000 units/kg body weight, intraperitoneal injection) to adult male rats 24 h prior to subjecting their isolated hearts in a Langendorff perfusion to an in vitro protocol of ischemia (30 min) followed by reperfusion (45 min). This protocol therefore allowed chronic pretreatment with Epo, but removed any contribution by blood-borne factors and/or cells to cardiac recovery. Even in this relatively brief period of reperfusion, cardiac performance following ischemia/reperfusion in the absence of Epo was decreased. Despite having no effects on heart rate or coronary flow in the reperfused hearts, Epo improved left ventricular developed pressure by approximately threefold relative to hearts not pretreated with Epo. This was accompanied by less apoptotic, TUNEL-positive cells and lower activity of the pro-apoptotic caspase-3. Although these results suggest that Epo pre-exposure protected the heart against acute ischemic insult followed by reperfusion, at least two critical questions are raised.

First, can Epo given at, or after, the time of insult also offer protection?

Second, can the beneficial effects of Epo on both suppressing apoptosis and retaining cardiac function be sustained beyond these initial acute time points?

As outlined in the following paragraphs, studies published in 2003 have initially provided positive answers to these questions.

The study by Tramontano et al. [9], again in the adult rat, has supported the initial findings of Epo in cardioprotection. Epo (5000 units/kg body weight, intraperitoneal injection) was delivered immediately following the ligation of the left anterior descending coronary artery. This avoided any effects of Epo that required prolonged treatment prior to the insult but like the study by Cai et al. [7], the effects following reperfusion were only evaluated acutely. After 60 min of ligation, the hearts were examined and the numbers of apoptotic, TUNEL-positive cells were significantly decreased in the Epo-treated hearts. Most TUNEL-positive cells in the control hearts were identified as myocytes and only occasionally endothelial cells or leukocytes so Epo appeared to prevent the death of the cardiac myocytes. Whilst no functional data on the performance of these hearts was reported, acute protection by Epo was at least partially via the maintenance of cell viability. Importantly, Epo was protective when administered after the insult and without pretreatment. This is particularly important if Epo is to be used to protect or salvage the heart post-infarction.

This raises the question of how long after the insult Epo treatment can be effective, i.e., what is the therapeutic window during which Epo can be used to save the heart? It also leaves open the question of how long the beneficial effects of Epo might last. Whilst there has not been any systematic study to evaluate Epo's therapeutic window, studies are already demonstrating longer term effects of Epo.

Parsa et al. [8] have evaluated the actions of Epo (5000 units/kg body weight, intraperitoneal injection) in adult rabbit heart. Epo was delivered at the time of coronary artery ligation and its effects studied at 3 days. Cardiac function was measured in vivo by micromanometry both under basal conditions and in response to β-adrenergic stimulation. Whilst Epo-treated hearts did not completely retain global function, peak left ventricular pressures and left ventricular relaxation were significantly improved when compared to non-Epo-treated animals subjected to infarct. Closer examination of the area at risk showed that Epo decreased the area of infarcted tissue. This was supported by the observation of lower numbers of TUNEL-positive cells in the left ventricular sections sampled 6 h after infarction in these Epo-treated animals.

Calvillo et al. [6] and Moon et al. [10] have also followed recovery in vivo for longer periods of time following Epo treatment. These studies have examined adult male rats. Calvillo et al. delivered Epo (5000 units/kg body weight, intraperitoneal injection) in two protocols. First, the effects of Epo pretreatment were evaluated by administration at 24 and 0.5 h prior to a 30-min ligation of the left anterior descending coronary artery. In the alternative protocol, Epo treatment was initiated at the time of reperfusion when the suture was removed [6]. In both protocols, animals subsequently received repeat doses of Epo every 24 h for the following 7 days, then haemodynamic measurements were made and hearts examined histologically. Cell death was measured together with cardiac myocyte cross-sectional area as an estimate of the hypertrophic response following increased workload. These studies showed less cardiac myocyte loss and a smaller increase in myocyte size in those animals treated with Epo prior to ischemia/reperfusion. These differences appeared sufficient to normalise haemodynamic function, specifically with ventricular wall stress remaining normal. Similar protection was also observed when Epo treatment was initiated at the time of reperfusion. This emphasises that pre-treatment with Epo was not essential for cardioprotection.

The study by Moon et al. [10] extends these conclusions in three ways. First, only a single dose of Epo (3000 units/kg body weight, intraperitoneal injection) rather than repeated Epo doses was required for protection. Second, Epo was cardioprotective after permanent ligation and so could protect against a more severe insult. Third, the effects on the heart were assessed at 1, 4 and 8 weeks following infarction, thereby beginning to address longer-term consequences of Epo administration. Again the effects of Epo were significant. Not only was there 50% reduction in the apoptotic cells in the area at risk at 24 h, but after 8 weeks the infarct size was 15–25% of the size of that seen in animals not treated with Epo. Thus, infarct expansion appeared to be attenuated by Epo treatment. Along with decreased damage, there were lesser changes in the left ventricular size, and repeated echocardiography over an 8-week period showed less functional decline of the Epo-treated animals. Thus, the left ventricular remodelling following coronary artery ligation that can result in a dilated thin-walled heart was abrogated by a single dose of Epo.

In all of these studies, it would appear that the initial actions of Epo to reduce apoptotic cell death were major contributors to the reduction in initial infarct areas and would thus decrease left ventricular dilatation. It is still not clear whether the high doses of Epo used in these cardioprotection studies, presumably chosen to directly parallel the neuroprotection studies, are needed when there is no equivalent of the blood–brain barrier to be overcome in cardiac protection. Presumably establishing a minimal effective dose will allow better estimates of the costs and benefits of Epo treatment following infarction.

## 6. Conclusions and future directions

Accumulating evidence suggests that the therapeutic benefits of Epo administration could be far more wide

reaching than initially anticipated. As discussed in the preceding sections, the actions of Epo to attenuate apoptotic cell death are likely major contributors to its cardioprotective actions. In this context, it should be noted that any additional wall stress from the loss of myocytes is likely itself a trigger for additional late apoptosis [87]. Therefore, the actions of Epo to prevent cell death in the very earliest stages of infarction could readily translate into more substantial effects to abrogate infarct expansion.

As more studies are undertaken to evaluate the actions of Epo, it should become clearer whether additional effects of Epo contribute to cardioprotection. Apart from possible anti-inflammatory properties as seen in Epo-treated brain tissue [20] or the effects of Epo to enhance myocyte contractility [88], Epo can stimulate neuronal and epithelial progenitor cell proliferation [39,60]. It remains possible that Epo will stimulate the development and mobilisation of immature non-differentiated stem cells into ischemic area of the myocardium. Whilst this is a slower acting action, it has the potential for prolonged protection with the possibility of substantial cell repopulation of the damaged tissue. There also appears to be the ability of Epo to act indirectly together with retinoic acid to support myocardial proliferation at least in the context of the intact embryonic heart [89]. Similar pro-proliferative actions have also been reported for cultured neonatal myocytes [90]. Such studies reveal a broader range of Epo actions for exploitation in future strategies that aim to improve cardiac recovery following insult. Epo may therefore, through its multiple actions on the cells of the cardiovascular system, rapidly join the arsenal of approaches directed against cardiovascular diseases.

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