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Review Article

Cardioprotective effects of erythropoietin: a journey from the bedside back to the bench

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Abstract

Recombinant human erythropoietin (rHuEPO) has been used for 15 years for the clinical management of anemia. Mitigation of chronic anemia has led to improved cardiac function, which served as a clinical indication for chronic EPO therapy in renal failure and congestive heart failure patients. Although it was originally believed that EPO acted solely on hematopoietic cells, recent evidence suggests additional non-hematopoietic effects involving direct actions in target tissues. For example, in animal models, a single dose (1,000-5,000 U/kg) administered around the time of myocardial infarction or reperfusion after ischemia can have a profound therapeutic effect on cardiac function independent of hematocrit. This direct cardioprotective effect of EPO appears to limit infarct size by preserving myocardium in the ischemic zone, leading to enhanced cardiac contractile function and increased inotropic reserve. Thus, a window of therapeutic opportunity may exist where a single dose of EPO following or in anticipation of an ischemic cardiac event may offer acute protection as well as long-lasting benefit, through preservation of viable myocardium during or after ischemic events.

Introduction

Despite significant research efforts, cardiovascular disease remains the greatest unsolved public health challenge. Erythropoietin (EPO) has been used in cardiovascular medicine for many years and has progressed from an agent used to manage chronic anemia to an agent associated with improvement in myocardial functional status, and to an agent that can mediate direct cardioprotective effects. Initially, research focused on the direct effect on hematopoiesis and correction of anemia. Interestingly, normalization of hematocrit and hemoglobin in mildly anemic patients with heart failure has shown potential therapeutic benefit on left ventricular (LV) function, a reduction in hospitalization days and, more importantly, an increase in quality of life (1-3). More recently, an accumulating body of experimental evidence has revealed a role for EPO and EPO receptor (EPO-R) signaling in modulating the physiological responses to various forms of tissue injury. In this review, we discuss the current use of EPO in cardiovascular diseases and its prospective applications for future clinical trials as a novel cardioprotective agent.

Physiology and pharmacology

Mammalian cells express certain stress proteins in response to ischemia (4). As an example, the expression of hypoxia-inducible factor (HIF)-1α increases logarithmically as cellular oxygen concentration decreases (5). To date, ischemic or pharmacological preconditioning remains the most reproducible and effective mechanism for the acquisition of ischemic tolerance and has important therapeutic implications. Effects downstream of increasing levels of HIF-1α include upregulation of

various proteins that mediate adaptive responses to hypoxia/ischemia, most notably EPO, the subject of this review.

The human EPO gene was cloned in 1983 and the introduction of rHuEPO in 1989 marked a significant advance in the management of chronic anemia (6). Erythopoietin is primarily synthesized by peritubular cells in the corticomedullary border of the kidney (4, 7), and regulates erythropoiesis by promoting the survival and proliferation of erythroid precursor cells (4). rHuEPO has a direct effect on hematopoiesis, reflected by increased hemoglobin levels which thereby increases the tissue oxygen supply (8).

While the kidneys are the predominant source of endogenous EPO production, a variety of other tissues have also been associated with EPO expression (9, 10). Further, studies have described diverse biological effects for EPO in various nonhematopoietic tissues that express functional EPO-R, such as the brain (11), retina (12), vascular smooth muscle (13), skeletal muscle (14), endothelial cells (15, 16) and heart (17-20). Other peptides can also stimulate the EPO-R, such as the novel erythropoiesis-stimulating protein (NESP), a therapeutic alternative to rHuEPO designed and expressed using recombinant DNA technology (21). NESP binds to the EPO-R, although with reduced affinity. The major benefit is a 3-fold increase (approximately 25 h) in the serum half-life over rHuEPO.

The EPO-R is a transmembrane receptor belonging to the type I cytokine receptor superfamily which homodimerizes upon activation (6, 22). Outside erythrocytes, the EPO-R is widely distributed, including in several cardiovascular cells such as endothelial cells (15), smooth muscle cells, cardiomyocytes (17, 18) and cardiac fibroblasts (18). The paradigm for EPO signaling has been delineated in numerous cell lines (23, 24). Hematological literature suggests that EPO is the primary regulator of erythropoiesis, and promotes the proliferation and survival of erythroid cells by preventing immature erythroblasts from undergoing apoptotic death (22). In general, an EPO-EPO-R interaction triggers specific intracellular protein kinase cascades, including the stress-responsive Janus-associated kinases (JAKs), which leads to activation of STAT (signal transducer and activator of transcription) transcription factors (23, 25). EPO-R signaling can also include activation of phosphatidylinositol 3-kinase (PI3-kinase)/Akt and mitogen-activated protein kinase (MAPK) pathways (23, 24).

Studies performed on immortalized human cell lines suggest that cell proliferation is regulated mainly by activation of MAPKs or the JAK2/STAT5 pathway, and inhibition of apoptosis is mediated primarily by activation of the PI3-kinase/Akt axis with a contribution from JAK2/STAT5 activation (23, 24). In the latter case, phosphorylated JAK2 triggers the activation of STAT5 protein, which translocates into the nucleus, binds to specific DNA response elements and induces a cascade of cellular responses, including the upregulation of antiapoptotic genes such as *bcl-2* and *bcl-*X_L (23, 24).

As a hematopoietic agent, EPO shows dose-dependent efficacy at up to 1,800 U/kg. There are no further increases in the reticulocyte response at 2,400 U/kg and no adverse effects at either of these doses (6, 26). As detailed below, potential cardioprotective doses may be higher (3,000-5,000 U/kg as a single dose) and these doses appear to increase circulating reticulocytes by day 3-5 (6, 17). Importantly, no LD_{50} for EPO has ever been reported (26, 27).

Role of EPO in cardiac development

Erythropoietin and the EPO-R are present in human fetal tissue (9). Although the heart produces minor amounts of EPO, the EPO-R is abundantly expressed in the myocardium as gestation progresses, suggesting its presence in adult cardiac tissue (9). Recently, van der Meer *et al*. and our laboratory described EPO expression in numerous cell lines that comprise the adult heart, including endothelial cells, fibroblasts and, to a lesser extent, cardiomyocytes (18, 28).

Experiments with EPO and EPO-R homozygous (–/–) knockout mice indicate that a loss of EPO signaling can lead to profound effects on the developing heart, as there is a recurring phenotype of ventricular hypoplasia purportedly due to a reduction in the number of proliferating cardiac myocytes in the ventricular myocardium (29). In addition to defects in cardiac morphogenesis, both EPO and EPO-R knockout mice exhibit abnormalities in the vascular network, and increased apoptosis in endocardium and myocardium (29, 30). In further experiments, Wu *et al*. found that EPO acts as a mitogen in isolated cardiomyocytes from $EPO^{-/-}$ and wild-type mice, but it had no effect in cells from $EPO\text{-}R^{-/-}$ mice (29). This indicates the importance of the EPO-R in nonhematopoietic cells.

Furthermore, Stuckmann *et al.* showed that blockade of either retinoic acid or EPO signaling from the chick epicardium inhibited cardiomyocyte proliferation and survival. Interestingly, the blockade of cardiomyocyte proliferation following administration of a retinoic acid antagonist can be rescued by exogenous EPO, and *vice versa* (31). These data are consistent with the findings of Wu *et al*. noted above (29). Collectively, these studies suggest that EPO and its receptor stimulate cardiomyocyte proliferation, at least during fetal stages.

First stop...cardiac effects of EPO in dialysis patients

Over the last 15 years, the use of rHuEPO has become widespread in the treatment of anemia secondary to end-stage renal disease. More than a decade ago it was shown that dialysis patients with significant coronary artery disease who were treated chronically with rHuEPO exhibited a reduction in exercise-induced myocardial ischemia (32). In addition to a direct effect on myocardial oxygenation through a rise in hemoglobin,

EPO lowers myocardial oxygen consumption by decreasing cardiac output and cardiac workload (33). Numerous studies have investigated the effects of normalizing hemoglobin in hemodialysis patients with suboptimal levels employing chronic rHuEPO supplementation (34). Patients with higher hemoglobin levels show a significantly reduced LV end-diastolic diameter by echocardiography (34), which appears to ameliorate LV hypertrophy (35). Echocardiographic findings in hemodialysis patients with severe anemia (hematocrit < 26%) treated with rHuEPO to elevate hematocrit levels above 30% showed a reduction in LV end-diastolic and end-systolic diameter, as well as LV end-diastolic and end-systolic volume. Ejection fraction, fractional shortening and the velocity of circumferential fiber shortening improved in the majority of treated patients (35).

Conversely, a Canadian multicenter trial failed to show that normalization of hemoglobin in patients with asymptomatic cardiomyopathy leads to regression of concentric LV hypertrophy or LV dilatation (36). Hayashi *et al*. studied the effects of preemptive rHuEPO in predialysis patients (*i.e*., patients who had not manifested severe LV hypertrophy at study inception). In patients with partially corrected anemia (30%), they observed a trend towards a reduction in LV hypertrophy, whereas in patients with normalized hematocrit (40%), this decrease appeared to be statistically significant (37).

Clinical data indicate that in the later phases of cardiac disease, when severe LV hypertrophy or LV dilatation has already manifested, the beneficial effects of anemia correction appear limited (36). Fortunately, these studies indicate that preemptive/early rHuEPO treatment abrogates the progression of LV hypertrophy (37). An explanation for EPO's limited effects in advanced cardiac disease could be the irreversible structural changes that occur, such as interstitial fibrosis (38).

The effect of rHuEPO on LV hypertrophy appears to be dependent upon the degree of anemia prior to initiation of therapy. The Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin beta (CREATE) trial will investigate the effect of early anemia correction on cardiovascular risk reduction in patients with renal impairment not yet on renal replacement therapy (39).

Overall, while controversy exists (40), consensus in the clinical literature indicates that EPO treatment of chronic anemia improves ejection fraction, reduces cardiac work, prevents ventricular dilatation, reduces congestive heart failure (CHF) exacerbations, reduces hospitalization and improves exercise tolerance, cognitive function and overall quality of life (41, 42). Unfortunately, once discontinued, these parameters apparently return to their pretreatment values within 1 year (42).

Second stop...EPO effects in heart failure patients

The beneficial results of chronic anemia management in end-stage renal disease led to an examination of a potential role for EPO therapy in the management of chronic anemia associated with congestive heart failure (CHF). Although plasma EPO levels are increased in patients with CHF, they are still insufficient to counterbalance the decreased hemoglobin levels found in anemic CHF patients (43).

Researchers at Tel Aviv Medical Center in Israel found that combination therapy with subcutaneous rHuEPO and intravenous iron was associated with improvement in cardiac function. The study enrolled 32 patients with severe CHF (New York Heart Association [NYHA] class of III or greater), with an LV ejection fraction of 40% or less and hemoglobin levels between 10.0 and 11.5 g/dl. These patients were randomized to receive rHuEPO and supplemental iron or placebo. Over about an 8-month period (mean hemoglobin increase to 12.9 g/dl), the treatment group (n=16) showed an improvement of 42% in NYHA class, while the control group worsened by 11%. Echocardiographic findings documented a 5.5% increase in LV ejection fraction in the treatment group, compared to a decrease of 5.4% in the control group. From a quality-of-life and medical economics standpoint, the number of hospitalization days decreased by 79% in the treatment group while it increased by 57.6% in the control group (1).

In a follow-up study, Silverberg *et al.* examined the effects of EPO treatment in diabetics and nondiabetics with severe CHF and mild to moderate renal failure. The authors found that correction of mild anemia (hemoglobin 9.5-11.5 g/dl) was beneficial in this patient population as well. In both diabetics and nondiabetics, the NYHA functional class improved by 34.8% and 32.4%, respectively, while LV ejection fraction improved by 7.4% and 11.5%, respectively. Along with improved cardiac function, treated patients exhibited a reduction in the number of hospitalizations, a reduction in diuretic requirements and overall improved quality of life (2).

Similar results were obtained in another study at Columbia Presbyterian Medical Center in New York involving 26 anemic patients with CHF randomized to receive rHuEPO or placebo for 70 days. Twenty-six patients with moderate to severe CHF (mean hemoglobin 11.0 g/dl) were randomized to receive either rHuEPO (15,000-30,000 IU/week) or placebo for 3 months. The treatment group exhibited significantly enhanced peak oxygen consumption (15.5%) and increased exercise duration (11.3%), resulting in an increased overall exercise capacity. This improvement correlated with a subjective improvement in the quality of life as per the Minnesota Living With Heart Failure Questionnaire. As a comment on EPO's side effect profile, the authors state that no effect on blood pressure was noted (3).

Despite the small sample size and lack of randomization for some of the above studies, the overall results strongly suggest an important role for rHuEPO (and supplemental iron) in the correction of even mild anemia in CHF, as there are reports of significant improvement in functional class, a reduction in hospitalizations, an increase in LV ejection fraction and an increase in exercise capacity. Certainly these results warrant larger

randomized trials in order to delineate the link between anemia and CHF and, importantly, these are ongoing.

Third stop...back to the bench

While the past 10 years have seen a clinical benefit in end-stage renal disease and CHF patients with respect to cardiovascular morbidity and mortality, work from several laboratories suggests that EPO may exert direct effects on cardiac myocytes. Although the clinical data on the effect of chronic rHuEPO therapy on LV structure and function may be attributed to a hematopoietic effect and the mitigation of physiological consequences of chronic anemia, expanding basic scientific research suggests that EPO plays a major role in nonerythropoietic cardiovascular tissues as an angiogenic, antiapoptotic, and possibly antiinflammatory cytokine agent. These direct effects were initially uncovered by studying the mitogenic effect of rHuEPO on fetal cardiomyocytes and development (see section on Role of EPO in cardiac development). Some of this exciting data is reviewed below.

Endothelial cells and angiogenesis

Stimulation of cultured endothelial cells with rHuEPO results in cell proliferation and differentiation into vascular structures (16). In human myocardial endothelial cells, EPO actually exhibits angiogenic potential comparable to that of vascular endothelial growth factor (VEGF), implying a role for rHuEPO in vasoproliferative processes (44). Further evidence is provided by EPO- and EPO-R-deficient transgenic mice, which develop dilated and independent vascular clumps in the myocardium instead of interconnected, fine vascular networks (29). EPO's role in angiogenesis should not be surprising since VEGF and EPO are both regulated by HIF-1α (22).

A recent paper from Scarabelli *et al.* showed that in the early stages of myocardial reperfusion, apoptosis is first seen in endothelial cells and then spreads to surrounding cardiomyocytes, suggesting reperfusion-induced release of proapoptotic mediators from endothelial cells (45). As the EPO-R in the heart is also expressed on endothelial cells (15), EPO treatment may prevent apoptosis in endothelial cells during reperfusion and thereby protect the myocardium and preserve vascular flow.

Myocyte apoptosis and preservation of area at risk

Apoptosis has been implicated as a key mechanism that contributes to the loss of cardiomyocytes in CHF (46) and ischemic injury (47). Saraste *et al*. observed apoptotic cardiomyocytes, particularly in the border zones of the infarcted myocardium, comparable to the penumbra in brain ischemia (47). This tissue, also known as the area at risk (48), is where EPO is thought to have the principal protective effect in the ischemic heart (17, 18). The data to date suggest that EPO administration may mimic ischemic preconditioning by limiting infarct size within the area at risk. In fact, ischemia/reperfusion (I/R) itself can activate pathways associated with EPO-EPO-R signaling in murine myocardium, including activation of JAK1, JAK2, STAT1 and STAT3 (49). Cai *et al.* also showed that HIF-1α is upregulated after cardiac I/R, which may also implicate EPO production in the heart to possibly act in a self-protective pathway or mechanism (50).

Over the last 2 years, numerous laboratories including our own have conducted *in vitro* and *in vivo* experiments to investigate the effect of rHuEPO on the cellular components that comprise the myocardium, as well as to assess the resultant physiology (see Table I). Administration of EPO has been associated with a 30-50% reduction in apoptotic nuclei in the post-myocardial infarction (MI) or post-I/R heart *in vivo* (17, 18, 20, 53). rHuEPO reduced cardiomyocyte loss over a 7-day period and attenuated the reactive hypertrophy of surviving cardiomyocytes in a rat model (20). This apparent direct cardioprotective effect of EPO appears to limit infarct size by preserving myocardium in the ischemic area at risk. The preserved myocardium results in improved cardiac contractile function and increased inotropic reserve even after MI. For example, β-adrenergic density and signaling are similar in noninfarcted LV tissue from control and EPO-treated hearts (17), suggesting that this increase in inotropic reserve is simply attributable to more viable

Table I: Summary of published reports on direct cardioprotective effects of rHuEPO: basic scientific results.

Investigator(s)	Reference	Outcome measures
Wright *et al.* (2004)	51	31% improvement in LVDP recovery after I/R, 20% increase in ATP levels during reperfusion
Parsa *et al*. (2004)	18	50% decrease in apoptotic nuclei during reperfusion, 50% reduction in LVEDP during reperfusion
Parsa *et al*. (2003)	17	50% decrease in apoptotic nuclei during ischemia, 23% reduction in infarct volume in AAR
Moon *et al*. (2003)	27	50% decrease in apoptotic nuclei during ischemia, 75% reduction in infarct volume
Cai and Semenza (2003)	19	50% improvement in LVDP recovery after I/R, benefit eliminated by wortmannin
Tramontano *et al*. (2003)	52	32% decrease in apoptotic nuclei during ischemia, benefit eliminated by LY-294002
Cai *et al*. (2003)	50	36% improvement in LVDP recovery after I/R
Calvillo *et al*. (2003)	20	Normalized LVEDP and wall stress 1 week after infarction

myocardium. Some groups also suggest that the improved cardiac function seen in the ischemic heart after EPO administration is due to improved coronary flow (53).

The main downstream signaling pathway that appears to be responsible for the increased myocyte survival attributed to EPO is the PI3-kinase/Akt pathway. In a cardiomyoblast cell line, EPO increased the activity of Akt under both normoxic and hypoxic conditions, while LY-294002, a specific PI3-kinase inhibitor, decreased EPO-stimulated Akt activity and also abrogated the anti-apoptotic effect of EPO (52). Furthermore, Akt inhibition by addition of the PI3-kinase inhibitor wortmannin reversed EPO-mediated protection after hypoxia (18, 19). Interestingly, the ERK (extracellular signal-related kinase)/MAPK inhibitor PD-98059 did not alter EPO-mediated myoblast survival (17). Cai *et al.* showed that the increased recovery of LV developed pressure and improved coronary flow rate, as well as reduction in LV end-diastolic pressure (LVEDP), in rHuEPO-treated hearts after I/R were completely blocked by coadministration of wortmannin (19). Thus, PI3-kinase may be involved in the pharmacological preconditioning effects of EPO to protect the ischemic heart.

Other data have demonstrated an involvement of EPO in improved maintenance of high-energy phosphates. ^{31}P nuclear magnetic resonance (NMR) spectrosocopy revealed that EPO administration was associated with preservation of ATP levels in the ischemic myocardium (51). The changes in intracellular pH and high-energy phosphate content mechanisms have been implicated in the myocardial protection afforded by ischemic preconditioning (54). Similar findings were encountered in response to pretreatment of the reperfused rat heart with rHuEPO. Erythropoietin administration modulates high-energy phosphate levels in perfused hearts, leading to reduced ATP depletion during ischemia, suggesting that preservation of high-energy phosphates may contribute to improved contractile recovery in EPO-treated hearts (51).

Importantly, cell death and infarct expansion occur primarily, although not exclusively, during the first 3 days after ischemic insult (55), *i.e.,* well before the expected maximum increase in red blood cell mass in response to EPO administration (17). Unlike the clinical models cited previously that employ chronic EPO therapy, the above animal studies employed a single high dose (1,000-5,000 U/kg) either prior to or simultaneous with ischemia. With this single dose administered around the time of MI or reperfusion, EPO has positive beneficial effects on infarct size, myocyte apoptosis and cardiac function prior to any rise in hematocrit.

Fibroblasts and remodeling

Another cell in the heart that is critical to ventricular remodeling after an insult is the cardiac fibroblast. The cardiac fibroblast can participate in postischemic LV remodeling in a variety of ways, including the release of several cytokines, chemokines and growth factors. Since cardiac fibroblasts constitute the most numerous cell type in the heart (56), and actively participate in postischemic remodeling, the response of cardiac fibroblasts to EPO probably represents an important process in postischemic inflammatory pathobiology. Interestingly, our laboratory has shown that EPO-R expression in cardiac fibroblasts is much higher than levels exhibited in ventricular myocytes (18). Thus, the direct cardioprotective effect of EPO may involve signaling pathways stimulated in cardiac fibroblasts.

Mechanistic signaling studies in cardiac fibroblasts indicate that the JAK2/STAT3 pathway appears to be the principle pathway stimulated by EPO, although Akt and ERK/MAPK pathways are also activated (18). Interestingly, ERK/MAPK activation by EPO can be inhibited by pertussis toxin (PTx), implicating a role for heterotrimeric G-proteins in EPO-R signaling (18). Conversely, JAK/STAT and Akt activation are PTx-insensitive, suggesting at least two distinct signaling cascades in EPO-stimulated fibroblasts (18). The role that these distinct EPO-EPO-R signaling pathways play in cardioprotection after ischemia *in vivo* is currently being investigated.

The downside

It is still important to note that the augmentation of hematocrit by EPO may not always translate into therapeutic gains when dealing with patients suffering from cardiovascular disease such as CHF. Erythopoietin administration can result in hypertension (57, 58), thrombotic complications (59), and potentially increase mortality rates in patients with ischemic heart disease (40). Some of these potentially limiting effects of EPO are reviewed below.

Thrombotic complications

Some reports suggest that EPO may have prothrombotic or platelet-activating effects. Erythropoietin increases the number of glycoprotein IIb/IIIa molecules on the platelet membrane and enhances thrombin-induced phosphorylation of platelet proteins (60). Reductions in protein C (40% from baseline) and S (50% from baseline) have been attributed to EPO (61). It is not clear whether the use of rHuEPO is associated with the development of thrombotic events (62). Studies evaluating the effect of rHuEPO on hemodialysis vascular access thrombosis have yielded conflicting results (59, 63). In a Canadian multicenter study, the overall rate of thrombosis was 0.28 per patient-year in rHuEPO-treated patients *versus* 0.05 per patient-year in controls (64). Many of these reports are small case series or comparisons with historical controls, so definitive conclusions are difficult to substantiate.

Table II: Possible etiologies of EPO-induced hypertension (see Refs. 26, 66, 67, 69).

Increased blood viscosity
Endothelin release
Upregulation of renin
Direct vasopressive effect on renal vessels
Elevation of cytosolic calcium in vascular smooth muscle cells
Alterations of nitric oxide synthesis

Hypertension

Increased blood pressure develops in 20-30% of renal patients treated with rHuEPO (65). Potentially, this may be due to other documented effects of EPO (see Table II), such as an increase in intracellular calcium (66), increase in endothelin release (26, 67), increase in tissue renin (26), as well as alternations in vascular tissue prostaglandin production (26). Recently, a transgenic mouse line (EPO-tg6) was generated that constitutively overexpresses human EPO cDNA 12-fold above basal levels in an oxygen-independent manner, with resultant hematocrit above 0.80 (70). These mice adapt to the high hematocrit and do not exhibit arterial hypertension or thrombotic events (69, 71). However, these mice suffer from severe diastolic dysfunction, which is typically associated with hypertrophic cardiomyopathy (72). Increased nitric oxide (NO) production (70) and decreased blood viscosity (69) appear to be adaptive mechanisms in these EPO-transgenic animals. Interestingly, these mice overexpressing EPO die rapidly from cardiovascular dysfunction when administered an NO synthase inhibitor (70). Importantly, our *in vivo* studies in the post-MI or post-I/R rabbit did not manifest any change in blood pressure with a single intravenous dose of rHuEPO (17, 18).

Summary and future directions

The future role of rHuEPO therapy in cardiovascular diseases seems promising. The recently described non-hematopoietic effects of EPO implicate it as a tissue-protective cytokine for organs that express the EPO-R, including the heart. Data indicate that brief exposure of hearts to EPO both *in vitro* and *in vivo* markedly enhances postischemic contractile recovery. Therapeutic exploitation is appealing but is hindered by the paucity of current knowledge regarding its mechanism, or by which cardiac cell type (myocyte *versus* fibroblast) is responsible for the cardioprotective effects of EPO. It is clear from the animal studies described that preservation of myocardial ATP levels during ischemia and proangiogenic properties appear integral to EPO's cardiac mechanism. Further basic research into the molecular mechanisms of the apoptosis cascade in cardiomyocytes and cardiac fibroblasts and the proangiogenic effects on endothelial cells will be necessary to delineate the merits of rHuEPO therapy. Moreover, a better understanding of the functional significance of PI3-kinase/Akt in EPO-mediated cardioprotection is needed, which may reveal novel, presently unidentified strategies to inhibit apoptosis in the myocardium. Overall, there may be a window of therapeutic opportunity where a single dose of EPO following myocardial ischemia may offer acute protection as well as long-lasting benefit, through preservation of viable myocardium.

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