www.nephropathol.com DOI:10.12860/JNP.2013.27 J Nephropathology. 2013; 2(3): 154-165

Journal of Nephropathology

Use of high-dose erythropoietin for repair after injury: A comparison of outcomes in heart and kidney

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ARTICLE INFO

Article type:
Review Article

Article history:
Received: 2 November 2012
Accepted: 25 December 2012
Published online: 1 July 2013

Keywords:
Erythropoietin
Cytokine
Ischemia-reperfusion
Cytoprotection
Heart
Kidney

ABSTRACT

Context: There is a need to define the exact benefits and contraindications of use of high-dose recombinant human erythropoietin (EPO) for its non-hematopoietic function as a cytokine that enhances tissue repair after injury. This review compares the outcomes from use of EPO in the injured heart and kidney, two organs that are thought, traditionally, to have intrinsically-different repair mechanisms.
Evidence Acquisitions: Directory of Open Access Journals (DOAJ), Google Scholar, Pubmed (NLM), LISTA (EBSCO) and Web of Science have been searched.
Results: Ongoing work by us on EPO protection of ischemia-reperfusion-injured kidneys indicated, first, that EPO acutely enhanced kidney repair via anti-apoptotic, pro-regenerative mechanisms, and second, that EPO may promote chronic fibrosis in the long term. Work by others on the ischaemia-injured heart has also indicated that EPO promotes repair. Although myocardial infarcts are made up mostly of necrotic tissue, many publications state EPO is anti-apoptotic in the heart, as well as promoting healing via cell differentiation and stimulation of granulation tissue. In the case of the heart, promotion of fibrosis may be advantageous where an infarct has destroyed a zone of cardiomyocytes, but if EPO stimulates progressive fibrosis in the heart, this may promote cardiac failure.
Conclusions: A major concern in relation to the use of EPO in a cytoprotective role is its stimulation of long-term inflammation and fibrosis. EPO usage for cytoprotection is undoubtedly advantageous, but it may need to be offset with an anti-inflammatory agent in some organs, like kidney and heart, where progression to chronic fibrosis after acute injury is often recorded.

Review Article

Implication for health policy/practice/research/medical education:
There is a need to define the exact benefits and contraindications of use of high-dose recombinant human erythropoietin (EPO) for its non-hematopoietic function as a cytokine that enhances tissue repair after injury. This review compares the outcomes from use of EPO in the injured heart and kidney, two organs that are thought, traditionally, to have intrinsically-different repair mechanisms.

Please cite this paper as: Gobe GC, Morais C, Vesey DA, Johnson DW. Use of high-dose erythropoietin for repair after injury: A comparison of outcomes in heart and kidney. J Nephropathology. 2013; 2(3):154-165, DOI: 10.12860/JNP.2013.27

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1. Context

Each year, acute kidney injury (AKI) from multiple causes affects many millions of people throughout the world (1). There are few successful therapies and approximately two-thirds of these patients will die. Ischemia-reperfusion (IR) is the most common cause of AKI and acute renal failure. Coronary heart disease is the most common cause of death throughout the world and is predicted to remain so until 2030 (2-4). Myocardial infarct (MI), or hypoxic-ischaemic heart, is a common cardiovascular disease and is responsible for substantial global morbidity and mortality. These examples of acute injury in kidney and heart have poor treatment options. Supraphysiological dosing with the drug recombinant human erythropoietin (EPO) is being trialled as a potential therapy for AKI and MI.

EPO ensures the numbers of mature erythrocytes that maintain correct haemoglobin levels and oxygenation of tissues. It acts via EPO receptors (EPORs) on the surface of erythrocyte progenitor cells (5), stopping their death by apoptosis and stimulating production of mature erythrocytes. This action promotes higher levels of haemoglobin and tissue oxygenation. Although EPO is typically administered to treat anaemia, the drug is also now known to possess useful non-hematopoietic functions, in particular as a cytokine that enhances repair after injury, acting via EPORs that are present on many cell types beyond those of the haemopoietic system (6). EPO administration ameliorates injury in kidney and heart (7-12), as well as injuries of the spinal cord, retinal neurons, skin, peripheral nerves, gut, lung, bowel, liver and pancreas (13-22). It acts via a combination of suppressed apoptosis and stimulated mitogenesis and cellular differentiation. Human trials for both AKI and MI are presently underway (23-30).

In this review, we discuss the role of EPO during wound healing and compare responses in both the kidney and heart. In addition to demonstrated beneficial effects, the review will also discuss evidence pertaining to potential long-term deleterious effects of EPO administered for acute injury, such as myofibroblast transformation, neoangiogenesis and chronic fibrosis. Future analyses of cellular and molecular pathways involved in EPO-stimulated tissue repair are warranted.

2. Evidence Acquisition

PubMed, Web of Science and Google Scholar were searched with key words relevant to use of EPO as a cytoprotectant, acute kidney injury, myocardial infarct, repair and fibrosis.

3. Results

Erythropoietin is a glycoprotein hormone that is best known for its regulation and stimulation of the production of red blood cells, often under the influence of hypoxia (6). Endogenous erythropoietin is produced primarily by renal cortical fibroblasts in response to hypoxia (31, 32). Whether synthesised as the endogenous hormone or delivered as a recombinant drug, EPO has an anti-apoptotic action on erythrocyte progenitors leading to stimulated erythropoiesis and production of mature erythrocytes. Other regulatory factors subsequently act to induce commitment and further maturation of the cells involved in the red cell lineage (33). The initiation of erythropoiesis enhances tissue oxygenation, which in turn regulates erythropoiesis via negative feedback (31).

EPOR is a type I cytokine receptor that is expressed on many cell types, including hematopoietic cells, endothelial and vascular smooth muscle cells, neurones, microglia, astrocytes and myelin sheaths, proximal tubule epithelium, renal cell carcinoma cells that originate from the

proximal tubular epithelium, glomerular mesangial cells, cardiomyocytes, myoblasts, enterocytes, mast cells, macrophages, myeloid cells, lymphocytes and megakaryocytes (34). In these cells, EPO exerts anti-apoptotic, anti-inflammatory, pro-regenerative, pro-angiogenic and cell differentiation effects. There is more recent evidence that EPO mediates tissue protection via another receptor, the common β-subunit heteroreceptor (βcR), that appears to have a physical and functional interaction with the EPOR (35).

Mechanistically, EPO modulates cellular signal transduction pathways to act in a cytoprotective fashion, particularly against hypoxia. There are several signalling pathways that have been described, but three main pathways still predominate: The classical pathway is where binding of EPO to EPOR initiates recruitment and phosphorylation of Janus kinase 2 (JAK2) (36), followed by recruitment of the transcription factor Stat5, which is then phosphorylated and dimerised by its interaction with JAK2. JAK2 then moves to the nucleus to upregulate Bcl-xL, an anti-apoptotic gene of the B-cell lymphoma (Bcl-2) gene family. The second pathway involves nuclear factor-κB (NF-κB), a survival protein induced by stress or inflammation. JAK2-dependent NF-κB activates an IκB kinase complex, and the release of NF-κB, which moves to the nucleus to stimulate expression of anti-apoptotic genes (37). In the third pathway, the EPO-EPOR interaction activates other survival kinases, such as mitogen-activated protein kinase (MAPK), phophatidylinositol 3-kinase and Akt to increase the rates of survival, proliferation and differentiation of the stimulated cells (38).

One of the usual concerns about the systemic use of EPO for cytoprotection is that, as well as tissue protection and stimulation of repair, there could be augmented stimulation of erythrocyte concentrations leading to increased blood viscosity and an increased risk of thrombosis (39). There are now some non-erythrocyte stimulating EPO agents, such as carbamylated EPO, that provide similar cytoprotection to EPO (40, 41) but do not stimulate erythropoiesis, thereby mitigating the risk of clotting and allaying concerns about abnormally-stimulated erythrocyte populations. Another concern about the use of EPO as a cytoprotective agent is that its anti-apoptotic actions may inadvertently stimulate cancer cell growth (42, 43). Finally, some inflammatory cell types with EPORs may be stimulated beyond their usefulness in the repair process. For example, cells central to fibrosis, like myofibroblasts and macrophages, may remain stimulated after repair has been completed, thereby contributing to progressive fibrosis. These concerns need careful analysis.

3.1. Wound healing – essential after injury

Wound healing is a normal and complex process that is initiated in response to tissue injury to restore the function and integrity of damaged tissues. Tissue injury stimulates the influx of inflammatory and vascular endothelial cells during wound healing, the latter because angiogenesis is an essential component of wound healing. Remodelling not only requires an increase in extracellular matrix (ECM) production, but also important changes in fibroblast to myofibroblast phenotypes. Central to repair after injury is the stimulation of granulation tissue, fibrous connective tissue that consists of macrophages, new vessels, myofibroblasts, and collagen or other similar binding proteins (44-47). Myofibroblasts are of varying lineages but can be thought of, simply, as fibroblasts that express smooth muscle cell proteins, such as α-smooth muscle actin. Expression of these proteins helps to provide tensile strength

to repairing tissue. In the heart, myofibroblasts have higher connexin-43 levels than fibroblasts, with the result that electrical coupling between isolated myocytes and myofibroblasts increases (48). This observation is important, because contacts between fibroblasts and cardiomyocytes in intact and healthy hearts are weak, and electrophysiological stimulation of myocytes may promote cardiac fibrosis.

EPORs have been immunolocalized to cells in granulation tissue: EPOR immunoreactivity was present in fibroblasts, macrophages, smooth muscle cells, and endothelium (47). Immunohistochemical analysis also revealed βcR immunoreactivity in granulation tissue fibroblasts, macrophages, smooth muscle cells, and endothelium (35). Expression of EPOR in wound macrophages was validated by performing double immunofluorescence for a macrophage marker and EPOR on the same granulation tissue section. EPO stimulates angiogenesis in granulation tissue (35), suggesting that the pro-repair effect of EPO is associated, at least in part, with its stimulatory actions on the proliferation and migration of endothelial cells during wound healing. Thus, EPO's ability to stimulate healing via production of a healthy granulation tissue is useful. The diminished healing response after administration of EPO antagonists provides evidence for the involvement of an endogenous EPO-EPOR system in the wound-healing cascade, that could be stimulated by delivery of high doses of the EPO drug (47). However, the conundrum of stimulated healing versus stimulated progressive chronic fibrosis must again be considered.

3.2. Inflammation - acute and chronic, and the role of the endothelium

Inflammatory responses in the heart and kidney after ischaemia-reperfusion injury can provide either beneficial or harmful results after injury. Healthy endothelium is central to inflammation in repair. Inflammatory cells themselves, however, produce endogenous cytotoxic factors and reactive oxygen species (ROS). The action of cells in the inflammatory response to ischaemia-reperfusion, such as neutrophils, lymphocytes, monocytes, macrophages, myofibroblasts and the vascular endothelium, can be modulated by EPO (48). However, the interrelationships between the responses to EPO, such as the needed anti-apoptotic effect on the damaged intrinsic functioning tissue, the beneficial stimulation of acute and chronic inflammatory cells, and the influence of a healthy endothelium in repair, need definition to develop or adapt therapies and promote best long-term outcome. The participating cells in inflammation that are likely to be most relevant to ischaemia-reperfusion-injured heart and kidney, and are possibly tissue-specific to these tissues, are listed below, along with questions that are relevant to this review.

3.2.1.Neutrophils

These short-lived acute inflammatory cells are a source of ROS. Whilst necessary for phagocytosis of necrotic tissue, their prolonged presence can be damaging by causing excess oxidative stress (49). In the heart, the invasion of neutrophils in the first 48h after infarction is marked. In contrast, in the kidney, the numbers of neutrophils are much more moderate. This may relate to the necrotic cells of the renal tubular epithelium being sloughed into the tubular lumen, within an often intact basement membrane, rather than the more open nature of a heart infarct. Does EPO minimise the detrimental effects of neutrophils in heart and kidney after IR?

3.2.2.Monocytes and macrophages

Monocytes are attracted to the site of injury via the vasculature, or may be activated to macro-

phages in situ. There are two macrophage phenotypes – M1 (damaging) and M2 (protective with reduced chemokine and inflammatory cytokine expression) (50). Does EPO modulate the balance of M1 and M2 macrophages in ischaemia-reperfusion-injured heart and kidney?

3.2.3. Myofibroblasts

In injured heart and kidney, there is likely to be a differing, tissue-specific, window of time when activated myofibroblasts are beneficial in repair, with their prolonged presence leading to excess fibrosis and scarring. Myofibroblasts express reparative cytokines, such as EPO (31), hypoxia-inducing factor (HIF)-2α, insulin-like growth factor-1 and its binding proteins (51, 52). We know that EPO causes excess stimulation of myofibroblasts in repair after AKI (53). Does it promote healing of the infarcted heart, where rapid building of a scar is central to repair?

3.2.4. Endothelium

Endothelial injury plays a key role in tissue damage of ischaemia-reperfusion (54). Stimulation of the endothelium and its progenitor cells has some benefits in allaying or repairing such injury. Does EPO act directly on endothelium, stimulating endothelial progenitor cells and angiogenesis, or act via a secondary cytokine/growth factor to stimulate a healthy endothelium and minimise inflammation in the ischaemia-reperfusion-injured heart and kidney?

3.3. The heart and kidney repair differently after injury

The adult heart and kidney are both affected by postnatal limitations on regeneration after repair: the cardiomyocytes of the heart are considered to be permanent, terminally differentiated, cell populations with limited ability for mitosis to replace necrotic cells (55); the kidney is limited by a lack of postnatal nephrogenesis, but the renal epithelial cells are considered stable, that is, they have the ability to enter the cell cycle for regeneration and repair if stimulated (56). Necrosis predominates in the heart infarct, whereas both apoptosis and necrosis occur in the ischaemia-injured kidney. Thus, inflammation may occur to a larger extent in MI compared with AKI. In part because of these characteristics, the heart and kidney repair differently after injury to their respective intrinsic working cells. The reasons are discussed in the following paragraphs.

3.3.1. Healing after acute MI

In acute MI, the lesion is typically characterised by a central necrotic core (the infarct) surrounded by a variably-sized region of potentially reversibly-injured cells at risk of further degeneration. Inflammatory reactions, and the build-up of self-perpetuating oxidative stress through increasing ROS, may significantly expand the original necrotic core. There is some evidence that apoptosis also plays a role. For example, overexpression of the anti-apoptotic gene Bcl-2 inhibits cardiomyopathy induced by prior MI (57) and deletion of the anti-apoptotic gene ARC exacerbates cardiomyopathy following aortic constriction (58). However, many papers claiming to identify apoptosis only have molecular analyses of apoptosis using whole tissue protein extract, such as expression of cleaved caspase-3, or localised labelling with the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labelling (TUNEL) assay for apoptosis without co-labelling of the cell type that has died. We believe apoptosis of inflammatory cells may occur at least as often as that of cardiomyocytes, and probably more so. Our recent analyses after MI (unpublished data), and that of others (59) after ischaemic injury, indicate that apoptosis of cardiomyocytes is a rare phenomenon in the adult heart (<0.1% in cardiomyocytes), and so an anti-apoptotic mechanism of EPO in cardiomyocytes is unlikely when EPO

is successful in treatment of MI.

The heart is formed by different types of cells, but mainly cardiomyocytes and fibroblasts. In some species, fibroblasts account for more than half of all heart cells and produce the ECM, which is composed of several types of collagen, as well as fibronectin (60). Cardiac fibroblasts and the ECM form a network to which the myocytes attach, and this helps maintain the structural integrity of the heart, while enabling fibroblasts to maintain mechanical forces on the myocytes. The bulk of healing in the infarcted heart is via building of a scar (61). Although it was previously considered that tissue fibrosis, in general, was the result of the activation and expansion of the pool of resident fibroblasts of mesenchymal origin, this concept has recently been challenged. Two hypotheses have been advanced: that resident cells of other lineages can differentiate into mesenchymal cells; or that a pool of bone marrow-derived fibrocytes accumulate in tissue in the presence of appropriate homing signals. Transdifferentiation of viable cardiomyocytes to myofibroblasts (epithelial-to-mesenchymal transdifferentiation, or EMT) may occur under the influence of profibrotic growth factors in repair (62).

After healing of a substantial sized infarct, heart failure is likely to occur via diffuse interstitial myocardial fibrosis. Heart failure is a common and disabling clinical syndrome that increases morbidity and mortality after repair of the MI (60). Given the critical role of the cardiac fibroblast in heart failure, it is important to understand the factors that determine the abundance and activity of these cells. Limiting expansion of the infarcted core (by an anti-fibrotic, anti-inflammatory mechanism) and repairing the necrotic core with minimal fibrosis is of immense benefit. EPO may promote such an outcome in the heart after MI.

3.3.2. Healing after AKI

AKI from multiple causes is characterised by death of the tubular epithelium and activation and expansion of the tubulointerstitium with inflammatory cells and ECM (63). The lesion may have extensive death (apoptosis and necrosis) and desquamation of the tubular epithelium into the lumen of the nephron, particularly in the outer stripe of the outer medulla and the thick ascending limb of the loop of Henle in the case of ischaemia-reperfusion injury. Thus, successful repair of kidney injury needs replacement of the tubular epithelium and minimal fibrosis. We have demonstrated that there is increased presence and activation of fibroblasts in the interstitial space and EMT in the tubular epithelium (64). Provided the basement membrane of the tubules is not disrupted, and there are some remnant tubular epithelial cells, we believe these cells regenerate by marked mitosis (65) and also perhaps mesenchymal-to-epithelial transdifferentiation.

The extent of recovery, and indeed progression to chronic kidney failure, depends on the severity of the insult. Compensatory structural changes in the kidney may cause the loss of renal function in focal damaged areas to be masked. Apoptosis and necrosis occur in the tubular epithelium (7, 8) and infiltrating leukocytes (neutrophils, monocytes, lymphocytes) appear rapidly as part of the acute repair process. Macrophages participate in removal of necrotic and apoptotic cells, and myofibroblasts are seen in the interstitial space, sitting closely around the basement membranes of damaged nephrons (52). Cellular proliferation is stimulated to replace the dead or damaged epithelial cells. Recovery after acute tubular necrosis may involve extensive remodeling of the tubules. Contrary to the long-held belief that only acute injury can be repaired, while ongoing chronic damage leads to progressive neph-

ron loss, evidence is emerging that some degree of renal remodeling occurs even in the presence of persistent structural changes (66). Limiting the extent of cell death and promoting proliferation of the tubular epithelium (these can be accomplished with EPO) while minimising extensive tubulointerstitial myofibroblast activation and fibrosis would be of immense benefit.

3.4. EPO therapy in heart

Cardiomyocytes have EPORs (71). If apoptosis of cardiomyocytes is a rare phenomenon in the adult heart after ischaemic injury (<0.1% in cardiomyocytes) (59), the anti-apoptotic mechanism of EPO in cardiomyocytes is unlikely to be acting in a substantial way when EPO is used successfully in treatment of MI. Yet studies in EPOR knockout mice have confirmed the role of EPORs in cardioprotection and have described the presence of apoptosis in MI (67). How then does EPO benefit the heart? EPO administration reduces the size of ischaemia-induced MI after ischaemia (68, 69). Gao et al (70) used the long-acting EPO, darbepoetin, delivered as late as 24h after the ischaemic insult. They found a significant reduction in the size of the MI, improved cardiac reserve and reported a decrease in cardiomyocyte apoptosis. Treatment with EPO improved left ventricular function and induced angiogenesis in a model of MI in rats and delayed EPO therapy reduced post-infarct cardiac remodelling (71, 72). Myocardial angiogenesis is promoted by EPO via protection from apoptosis of endothelial progenitor cells in the bone marrow, a direct stimulatory effect on myocardial endothelial cells and enhancement of the activity of endothelial progenitor cells (73). Systemic effects of high dose EPO delivery were considered by Kobayashi et al (74, 75). They therefore examined the effect of a local EPO-containing delivery system applied to the surface of an ischaemic infarct of rabbit heart. The infarct size was reduced and left ventricular remodelling improved long-term in the EPO-treated animals. These changes were associated with increased vascular endothelial growth factor concentrations, augmented angiogenesis, activation of the MAPK extracellular signal-regulated kinase (ERK) (presumably for stimulation of mitosis and cell differentiation), and increased levels of Akt and Stat-3 (74, 75). There was also evidence of metalloproteinase expression and reduction of fibrotic areas in the heart.

The clinical implications of these observations are important. The ability for EPO to provide protection even after the MI has occurred, its stimulation of angiogenesis and its influence on reducing fibrosis should improve patient outcomes. In MI patients, endogenous levels of erythropoietin increased after MI and remained elevated for some time after the injury (76, 77). There were concerns about the high dose bolus of EPO required for its cardioprotective effect because use of EPO is associated with polycythaemia, and significant risk of thromboembolic complications. Safety considerations of single high dose EPO were studied by Lipsic et al (69). The single bolus of 60,000 IU EPO delivered in patients with acute MI was not associated with significantly increased blood pressure and plasma haemoglobin levels, although plasma EPO concentrations increased 200-fold over normal levels. Endothelial progenitor cells increased in the EPO-treated patients. However, results seen in experimental animals have not, in general, translated well to human studies (78). Clinical trials of patients with acute MI are now in progress (erythropoietin in acute myocardial infarction [EAMI] NCT00149058 (26), ErythroPoetin in Myocardial Infarction [EPOMI] NCT00648089 (27); HEBEIII NCT00449488 (28); MAGIC Cell-5-Com-

bicytokine Trial NCT00501917; NCT00378352) (29, 30).

3.5. EPO therapy in the kidney

Administration of exogenous EPO in both in vitro and in vivo models of ischaemic AKI significantly hastened renal structural and functional recovery (6). In an in vitro model of ischaemia-reperfusion injury, treatment of human proximal tubule cells with 200IU/mL of EPO inhibited apoptosis in cells exposed to hypoxia with or without subsequent anoxia (7). Dosage at 400U/mL also stimulated cell proliferation in the same model. In an in vivo rat model of renal ischaemia-reperfusion injury, EPOR expression was well-maintained during the early phase, whereas endogenous EPO was unchanged or minimal. When rats were treated with EPO (5,000U/kg) at the time of ischaemia-reperfusion injury, tubular cell apoptosis was significantly decreased compared with vehicle-treated controls, particularly in the region of the hypoxia-sensitive proximal straight tubule (7, 8). The kidneys of EPO-treated animals also demonstrated enhanced tubular regeneration and decreased cast formation. Although haematocrit readings rose in EPO-treated animals compared with vehicle-treated controls, this increase was not statistically or clinically significant. Darbepoetin-α exerted comparable renoprotection compared with epoetin-α in ischaemia-reperfusion injury in rat kidneys, and the benefits were still apparent even when administration was delayed by 6h after onset of reperfusion (8). The results were confirmed in porcine and canine (79, 80) models of ischaemic AKI. Other models of AKI had a similar outcome. There were in fact few studies reporting adverse results, or those showing lack of improvement. However, Andratshke et al (81) reported that concomitant EPO administration significantly increased the degree of radiation-induced kidney damage in a C3H mouse model. The reasons for this were unclear but may relate to the predominant injury of endothelial cells in the radiation model. Mobilisation of endothelial progenitor cells by EPO may also protect against AKI .

We have investigated whether treatment of ischemia-reperfusion-induced AKI with EPO impacts on development of chronic kidney disease (manuscript submitted), (53). Using a rat model, EPO (1000 or 5000U/kg) or saline was delivered to rats subjected to bilateral 40-minute ischemia-reperfusion, and kidneys were studied at 4, 7 and 28 days post-surgery. In culture, renal tubular epithelial and fibroblast cells were subjected to hypoxia or hydrogen peroxide (1mM) with or without 200U/mL EPO. EPO ameliorated acute tubular damage, but at 28 days post-ischaemia-reperfusion with EPO, tubulointerstitial fibrosis was significantly greater (5000U/kg; $p<0.01$) or increased (1000U/kg; $p>0.05$). Interstitial myofibroblast numbers remained significantly increased over that seen in EPO animals ($p<0.01$). In culture, EPO also protected and activated fibroblasts, as well as protecting tubular epithelium, particularly with oxidative stress-induced injury. Thus, although EPO was cytoprotective of tubular epithelium in ischaemia-reperfusion-induced AKI, it may also have contributed to chronic kidney disease by overly stimulating resident interstitial fibroblasts.

Translation of the experimental results to human trials has started. Several clinical trials of EPO cytoprotection are underway in AKI (ERIN NCT00476619; ICU patients NCT00676234; post-cardiac surgery NCT00654992; renal transplantation NCT00425698) (6). Endre et al (24) performed a double-blind placebo-controlled trial (the EARLY-ARF trial) to study whether early treatment with EPO could prevent the develop-

ment of AKI in patients. Patients for treatment were selected after urinary levels of the proximal tubular brush border enzymes gamma-glutamyl transpeptidase and alkaline phosphatase were measured. They reported no difference in the incidence of EPO-specific adverse events or in the primary outcome between the placebo and treatment groups. Early intervention with high-dose EPO was, therefore, safe but EPO administration did not alter the outcome.

4. Conclusions

There is clear evidence that administration of EPO at or near the time of injury in experimental AKI and acute MI significantly improves recovery acutely, although the long-term effects have not been studied in detail. Beneficial cytoprotective effects exerted by EPO include inhibition of apoptosis, stimulation of mitogenesis and cell differentiation, mobilisation and differentiation of endothelial progenitor cells, angiogenesis and suppression of proinflammatory cytokine mediators. Major concerns relate to possible thromboembolic side effects of supraphysiological doses of EPO, as well as pro-neoplastic effects in cancer patients as a result of stimulation of angiogenesis and inhibition of apoptosis. Several randomised, controlled trials of EPO administration are underway for AKI and MI. One concern not given enough coverage in the research literature is stimulation by EPO of long-term inflammation and fibrosis. EPO administration for cytoprotection is undoubtedly advantageous, but it may need to be offset with an anti-inflammatory agent in some organs, such as kidney and heart, where progression to chronic fibrosis after acute injury is common.

Authors' contributions

GCG and DWJ contributed to the conception of the idea, literature search, drafting, and editing of the manuscript. CM and DAV contributed to literature search, editing and formatting of the manuscript. All authors critically reviewed and accepted the final version of the manuscript.

Conflict of interest

Professor David Johnson is a current recipient of a Queensland Government Health Research Fellowship. He has received consultancy fees, research funds, speaking honoraria and travel sponsorships from Jannsen-Cilag, Amgen, Pfizer and Roche. All other authors verify that they have nothing to disclose.

References

1. Wang HE, Muntner P, Chertow GM, Warnock DG. Acute kidney injury and mortality in hospitalized patients. Am J Nephrol. 2012;35(4):349-55.
2. Halim SA, Newby LK. Prognostic biomarkers in individuals with prevalent coronary heart disease. Dis Markers. 2009;26(5-6):265-71.
3. World Health Organization. Cardiovascular Diseases. 2012 [cited 2012 20 Novemebr 2012]; Available from: .
4. World Health Organization. Global status report on noncommunicable diseases. 2010.
5. Munugalavadla V, Kapur R. Role of c-Kit and erythropoietin receptor in erythropoiesis. Crit Rev Oncol Hematol. 2005;54(1):63-75.
6. Johnson DW, Vesey DA, G.C. G. Erythropoietin protects against acute kidney injury and failure. Open Drug Discovery Journal. 2009;1:41-50.
7. Vesey DA, Cheung C, Pat B, Endre Z, Gobe G, Johnson DW. Erythropoietin protects against ischaemic acute renal injury. Nephrol Dial Transplant. 2004;19(2):348-55.
8. Johnson DW, Pat B, Vesey DA, Guan Z, Endre Z, Gobe GC. Delayed administration of darbepoetin or erythropoietin protects against ischemic acute renal injury and failure. Kidney Int. 2006;69(10):1806-13.
9. Weng S, Zhu X, Jin Y, Wang T, Huang H. Protective effect of erythropoietin on myocardial infarction in rats by inhibition of caspase-12 expression. Experimental and therapeutic medicine. 2011;2(5):833-6.
10. Lu MJ, Chen YS, Huang HS, Ma MC. Erythropoietin alleviates post-ischemic injury of rat hearts by attenuating

nitrosative stress. Life Sci. 2012;90(19-20):776-84.
11. Li XJ, Wang XW, Du YJ. Protective effects of erythropoietin on myocardial infarction in rats: the role of AMP-activated protein kinase signaling pathway. Am J Med Sci. 2011;342(2):153-9.
12. Sharples EJ, Thiemermann C, Yaqoob MM. Novel applications of recombinant erythropoietin. Curr Opin Pharmacol. 2006;6(2):184-9.
13. Hong Z, Hong H, Chen H, Wang Z, Hong D. Investigation of the protective effect of erythropoietin on spinal cord injury in rats. Experimental and therapeutic medicine. 2011;2(5):837-41.
14. Rong X, Mo X, Ren T, Yang S, Yuan W, Dong J, et al. Neuroprotective effect of erythropoietin-loaded composite microspheres on retinal ganglion cells in rats. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences. 2011;43(4):334-42.
15. Bader A, Ebert S, Giri S, Kremer M, Liu S, Nerlich A, et al. Skin regeneration with conical and hair follicle structure of deep second-degree scalding injuries via combined expression of the EPO receptor and beta common receptor by local subcutaneous injection of nanosized rhEPO. International journal of nanomedicine. 2012;7:1227-37.
16. Elfar JC, Jacobson JA, Puzas JE, Rosier RN, Zuscik MJ. Erythropoietin accelerates functional recovery after peripheral nerve injury. The Journal of bone and joint surgery American volume. 2008;90(8):1644-53.
17. Kao NR, Xenocostas A, Driman DK, Rui T, Huang W, Jiao X, et al. Recombinant human erythropoietin improves gut barrier function in a hemorrhagic shock and resuscitation rat model. The Journal of trauma. 2011;71(5 Suppl 1):S456-61.
18. Kakavas S, Demestiha T, Vasileiou P, Xanthos T. Erythropoetin as a novel agent with pleiotropic effects against acute lung injury. European journal of clinical pharmacology. 2011;67(1):1-9.
19. Cuzzocrea S, Mazzon E, Di Paola R, Patel NS, Genovese T, Muia C, et al. Erythropoietin reduces the development of experimental inflammatory bowel disease. J Pharmacol Exp Ther. 2004;311(3):1272-80.
20. Guneli E, Cavdar Z, Islekel H, Sarioglu S, Erbayraktar S, Kiray M, et al. Erythropoietin protects the intestine against ischemia/ reperfusion injury in rats. Molecular medicine. 2007;13(9-10):509-17.
21. Shawky HM, Younan SM, Rashed LA, Shoukry H. Effect of recombinant erythropoietin on ischemia-reperfusion-induced apoptosis in rat liver. Journal of physiology and biochemistry. 2012;68(1):19-28.
22. Choi D, Schroer SA, Lu SY, Wang L, Wu X, Liu Y, et al. Erythropoietin protects against diabetes through direct effects on pancreatic beta cells. J Exp Med. 2010;207(13):2831-42.
23. Song YR, Lee T, You SJ, Chin HJ, Chae DW, Lim C, et al. Prevention of acute kidney injury by erythropoietin in patients undergoing coronary artery bypass grafting: a pilot study. Am J Nephrol. 2009;30(3):253-60.
24. Endre ZH, Walker RJ, Pickering JW, Shaw GM, Frampton CM, Henderson SJ, et al. Early intervention with erythropoietin does not affect the outcome of acute kidney injury (the EARLYARF trial). Kidney Int. 2010;77(11):1020-30.
25. Prunier F, Biere L, Gilard M, Boschat J, Mouquet F, Bauchart JJ, et al. Single high-dose erythropoietin administration immediately after reperfusion in patients with ST-segment elevation myocardial infarction: results of the erythropoietin in myocardial infarction trial. Am Heart J. 2012;163(2):200-7 e1.
26. Erythropoietin in acute myocardial infarction (EAMI) NCT00149058. 2005.
27. EPOMI Study: ErythroPOietin in Myocardial Infarction NCT00648089. 2011.
28. Clinical Study to Examine the Effects of Erythropoietin on Left Ventricular Function After Acute Myocardial Infarction (HEBEIII) NCT00449488. 2009
29. MAGIC Cell-5-Combicytokine Trial NCT00501917. 2007
30. REVEAL: Reduction of Infarct Expansion and Ventricular Remodeling With Erythropoietin After Large Myocardial Infarction NCT00378352. 2012.
31. Bachmann S, Le HM, Eckardt KU. Co-localization of erythropoietin mRNA and ecto-5'-nucleotidase immunoreactivity in peritubular cells of rat renal cortex indicates that fibroblasts produce erythropoietin. J Histochem Cytochem. 1993;41(3):335-41.
32. Lacombe C, Da Silva JL, Bruneval P, Fournier JG, Wendling F, Casadevall N, et al. Peritubular cells are the site of erythropoietin synthesis in the murine hypoxic kidney. J Clin Invest. 1988;81(2):620-3.
33. Fisher JW. Erythropoietin: physiology and pharmacology update. Exp Biol Med (Maywood). 2003;228(1):1-14.
34. Johnson DW, Forman C, Vesey DA. Novel renoprotective actions of erythropoietin: new uses for an old hormone. Nephrology (Carlton). 2006;11(4):306-12.
35. Brines M, Grasso G, Fiordaliso F, Sfacteria A, Ghezzi P, Fratelli M, et al. Erythropoietin mediates tissue protection through an erythropoietin and common be-

ta-subunit heteroreceptor. Proc Natl Acad Sci U S A. 2004;101(41):14907-12.
36. Wojchowski DM, He TC. Signal transduction in the erythropoietin receptor system. Stem Cells. 1993;11(5):381-92.
37. Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-kappaB signalling cascades. Nature. 2001;412(6847):641-7.
38. Jelkmann W, Wagner K. Beneficial and ominous aspects of the pleiotropic action of erythropoietin. Ann Hematol. 2004;83(11):673-86.
39. Lippi G, Franchini M, Favaloro EJ. Thrombotic complications of erythropoiesis-stimulating agents. Seminars in thrombosis and hemostasis. 2010;36(5):537-49.
40. Leist M, Ghezzi P, Grasso G, Bianchi R, Villa P, Fratelli M, et al. Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science. 2004;305(5681):239-42.
41. Fiordaliso F, Chimenti S, Staszewsky L, Bai A, Carlo E, Cuccovillo I, et al. A nonerythropoietic derivative of erythropoietin protects the myocardium from ischemia-reperfusion injury. Proc Natl Acad Sci U S A. 2005;102(6):2046-51.
42. Hadland BK, Longmore GD. Erythroid-stimulating agents in cancer therapy: potential dangers and biologic mechanisms. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27(25):4217-26.
43. Li J, Vesey DA, Johnson DW, Gobe G. Erythropoietin reduces cisplatin-induced apoptosis in renal carcinoma cells via a PKC dependent pathway. Cancer Biol Ther. 2007;6(12):1944-50.
44. Gethin G. Understanding the inflammatory process in wound healing. Br J Community Nurs. 2012;Suppl:S17-8, S20, S2.
45. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. Expert Rev Mol Med. 2011;13:e23.
46. Inoue T, Croce K, Morooka T, Sakuma M, Node K, Simon DI. Vascular inflammation and repair: implications for re-endothelialization, restenosis, and stent thrombosis. JACC Cardiovasc Interv. 2011;4(10):1057-66.
47. Haroon ZA, Amin K, Jiang X, Arcasoy MO. A novel role for erythropoietin during fibrin-induced wound-healing response. Am J Pathol. 2003;163(3):993-1000.
48. Coleman T, Brines M. Science review: recombinant human erythropoietin in critical illness: a role beyond anemia? Crit Care. 2004;8(5):337-41.
49. Hoffman JW, Jr., Gilbert TB, Poston RS, Silldorff EP. Myocardial reperfusion injury: etiology, mechanisms, and therapies. J Extra Corpor Technol. 2004;36(4):391-411.
50. Wang Y, Harris DC. Macrophages in renal disease. J Am Soc Nephrol. 2011;22(1):21-7.
51. Johnson DW, Saunders HJ, Brew BK, Ganesan A, Baxter RC, Poronnik P, et al. Human renal fibroblasts modulate proximal tubule cell growth and transport via the IGF-I axis. Kidney Int. 1997;52(6):1486-96.
52. Yang T, Pat BK, Veset D, Johnson DW, Wei MQ, Gobe GC. Renal tubulointerstitial fibrosis: role of apoptosis of myofibroblasts in limiting pro-fibrotic factors. Transl Res. 2007;150:40-50.
53. Gobe GC, Vesey DA, Bennett N, Johnson, DW. Recombinant human erythropoietin reduces ischemic acute kidney injury but does not stop progression to chronic kidney disease. . Nephrology (Carlton). 2008;13 (3):A135 (Abstr).
54. Brodsky SV, Yamamoto T, Tada T, Kim B, Chen J, Kajiya F, et al. Endothelial dysfunction in ischemic acute renal failure: rescue by transplanted endothelial cells. Am J Physiol Renal Physiol. 2002;282(6):F1140-9.
55. Buja LM, Vela D. Cardiomyocyte death and renewal in the normal and diseased heart. Cardiovasc Pathol. 2008;17(6):349-74.
56. Larsson SH, Aperia A. Renal growth in infancy and childhood--experimental studies of regulatory mechanisms. Pediatr Nephrol. 1991;5(4):439-42.
57. Chatterjee S, Stewart AS, Bish LT, Jayasankar V, Kim EM, Pirolli T, et al. Viral gene transfer of the antiapoptotic factor Bcl-2 protects against chronic postischemic heart failure. Circulation. 2002;106(12 Suppl 1):I212-7.
58. Donath S, Li P, Willenbockel C, Al-Saadi N, Gross V, Willnow T, et al. Apoptosis repressor with caspase recruitment domain is required for cardioprotection in response to biomechanical and ischemic stress. Circulation. 2006;113(9):1203-12.
59. McGaffin KR, Zou B, McTiernan CF, O'Donnell CP. Leptin attenuates cardiac apoptosis after chronic ischaemic injury. Cardiovasc Res. 2009;83(2):313-24.
60. Arenal A, Hernandez J, Perez-David E, Rubio-Guivernau JL, Ledesma-Carbayo MJ, Fernandez-Aviles F. Do the spatial characteristics of myocardial scar tissue determine the risk of ventricular arrhythmias? Cardiovasc Res. 2012;94(2):324-32.
61. Dor V, Civaia F, Alexandrescu C, Montiglio F. The post-myocardial infarction scarred ventricle and congestive heart failure: the preeminence of magnetic resonance imaging for preoperative, intraoperative, and postoperative assessment. J Thorac Cardiovasc Surg. 2008;136(6):1405-12.

62. Reinecke H, MacDonald GH, Hauschka SD, Murry CE. Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. J Cell Biol. 2000;149(3):731-40.
63. Yang L, Humphreys BD, Bonventre JV. Pathophysiology of acute kidney injury to chronic kidney disease: maladaptive repair. Contributions to nephrology. 2011;174:149-55.
64. Pat B, Hughson MD, Nicol JL, Hoy WE, Gobe GC. A comparison of pathomolecular markers of fibrosis and morphology in kidney from autopsies of African Americans and whites. Virchows Arch. 2007;450(1):41-50.
65. Gobe G, Zhang XJ, Willgoss DA, Schoch E, Hogg NA, Endre ZH. Relationship between expression of Bcl-2 genes and growth factors in ischemic acute renal failure in the rat. J Am Soc Nephrol. 2000;11(3):454-67.
66. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest. 2006;116(2):288-96.
67. Wright GL, Hanlon P, Amin K, Steenbergen C, Murphy E, Arcasoy MO. Erythropoietin receptor expression in adult rat cardiomyocytes is associated with an acute cardioprotective effect for recombinant erythropoietin during ischemia-reperfusion injury. Faseb J. 2004;18(9):1031-3.
68. Bogoyevitch MA. An update on the cardiac effects of erythropoietin cardioprotection by erythropoietin and the lessons learnt from studies in neuroprotection. Cardiovasc Res. 2004;63(2):208-16.
69. Lipsic E, Schoemaker RG, van der Meer P, Voors AA, van VDJ, van GWH. Protective effects of erythropoietin in cardiac ischemia: from bench to bedside. J Am Coll Cardiol. 2006;48(11):2161-7.
70. Gao E, Boucher M, Chuprun JK, Zhou RH, Eckhart AD, Koch WJ. Darbepoetin alfa, a long-acting erythropoietin analog, offers novel and delayed cardioprotection for the ischemic heart. Am J Physiol Heart Circ Physiol. 2007;293(1):H60-8.
71. van der Meer P, Lipsic E, Henning RH, Boddeus K, van dVJ, Voors AA, et al. Erythropoietin induces neovascularization and improves cardiac function in rats with heart failure after myocardial infarction. J Am Coll Cardiol. 2005;46(1):125-33.
72. van der Meer P, Lipsic E, van GWH, van VDJ. Erythropoietin: from hematopoiesis to cardioprotection. Cardiovasc Drugs Ther. 2005;19(1):7-8.
73. Prunier F, Pfister O, Hadri L, Liang L, Del MF, Liao R, et al. Delayed erythropoietin therapy reduces post-MI cardiac remodeling only at a dose that mobilizes endothelial progenitor cells. Am J Physiol Heart Circ Physiol. 2007;292(1):H522-9.
74. Kobayashi H, Minatoguchi S, Yasuda S, Bao N, Kawamura I, Iwasa M, et al. Post-infarct treatment with an erythropoietin-gelatin hydrogel drug delivery system for cardiac repair. Cardiovasc Res. 2008;79(4):611-20.
75. Kobayashi H, Miura T, Ishida H, Miki T, Tanno M, Yano T, et al. Limitation of infarct size by erythropoietin is associated with translocation of Akt to the mitochondria after reperfusion. Clin Exp Pharmacol Physiol. 2008;35(7):812-9.
76. Namiuchi S, Kagaya Y, Ohta J, Shiba N, Sugi M, Oikawa M, et al. High serum erythropoietin level is associated with smaller infarct size in patients with acute myocardial infarction who undergo successful primary percutaneous coronary intervention. J Am Coll Cardiol. 2005;45(9):1406-12.
77. Ferrario M, Massa M, Rosti V, Campanelli R, Ferlini M, Marinoni B, et al. Early haemoglobin-independent increase of plasma erythropoietin levels in patients with acute myocardial infarction. Eur Heart J. 2007;28(15):1805-13.
78. Tilling L, Clapp B. Erythropoietin: a future therapy for failing hearts? Heart Fail Rev. 2012;17(3):475-83.
79. Kuriyama S, Tomonari H, Yoshida H, Hashimoto T, Kawaguchi Y, Sakai O. Reversal of anemia by erythropoietin therapy retards the progression of chronic renal failure, especially in nondiabetic patients. Nephron. 1997;77(2):176-85.
80. Forman CJ, Johnson DW, Nicol DL. Erythropoietin administration protects against functional impairment and cell death after ischaemic renal injury in pigs. BJU Int. 2007;99(1):162-5.
81. Andratschke N, Schnaiter A, Weber WA, Cai L, Schill S, Wiedenmann N, et al. Preclinical evaluation of erythropoietin administration in a model of radiation-induced kidney dysfunction. Int J Radiat Oncol Biol Phys. 2006;64(5):1513-8.