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Erythropoietin in cardiac ischemia

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Chapter 8

Protective effects of erythropoietin in cardiac ischemia: from bench to bedside

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Submitted

Abstract

Erythropoietin (EPO) is a hypoxia-induced hormone produced in kidneys that stimulates hematopoiesis in the bone marrow by inhibiting the apoptosis of erythroid precursors. However, recent studies demonstrated also important non-hematopoietic effects of EPO. Administration of EPO confers neuroprotection against stroke and other neurological disorders. An active EPO receptor is found also in the cardiovascular system, including endothelial cells and cardiomyocytes. In animal studies, treatment with EPO during ischemia/reperfusion in the heart has been shown to limit the infarct size and the extent of apoptosis. In the longer term, EPO may promote ischemia-induced neovascularization, either by stimulating endothelial cells in situ or by mobilizing endothelial progenitor cells from bone marrow. The effects of EPO in the ischemic heart support the concept of EPO as a pleiotropic, tissue-protective agent for other organs expressing the EPO receptor. We recently performed a first randomized clinical study showing safety and feasibility of EPO administration in patients with acute myocardial infarction. Future clinical studies are warranted to translate the beneficial effects of EPO from basic experiments to cardiac patients.

Erythropoietin

Erythropoietin (EPO) is a hematopoietic hormone produced primarily in kidneys in response to hypoxia ⁽¹⁾. Hematopoietic effects of EPO in the bone marrow are mediated by binding to a specific transmembrane receptor (EPO-R) which is expressed primarily by erythroid progenitor cells ⁽²⁾. The signaling by the EPO-R is initiated when it is dimerized by binding EPO ⁽³⁾, leading to activation of numerous intracellular pathways (PI3K/Akt, MAPK, STAT-5) associated with cell survival ⁽⁴⁾. Rather than directly stimulating the proliferation, EPO inhibits the apoptosis of erythroid precursor cells ⁽⁵⁾. By reducing the apoptosis of erythroid cells in bone marrow, EPO upregulates the number of mature red blood cells in circulation. Although only present in plasma at picomolar concentrations, EPO regulates the production of approximately 2.3 million cells per second ⁽⁶⁾.

Epoetin alfa is the recombinant (rhEPO) form of the endogenous cytokine, with equal biological activity ⁽⁷⁾. RhEPO was originally used only in management of anemia caused by chronic kidney disease ⁽⁸⁾. The indication for EPO treatment has broadened in the last few years also to include other forms of anemia where EPO deficiency is not causative. It is now widely used in anemic patients with myelodysplastic syndromes ⁽⁹⁾, cancer patients treated with chemotherapy ⁽¹⁰⁾, and as prophylactic treatment to reduce the need for transfusions before major surgery ⁽¹¹⁾.

In addition to the effects related to hematopoiesis, numerous experimental studies have recently demonstrated important non-hematopoietic functions of EPO, such as protection against ischemic injury in various tissues. This effect was observed independently of hematopoiesis stimulation, indicating EPO may play a role as a pleiotropic survival and growth factor ⁽⁶⁾.

This review summarizes the newly established non-hematopoietic properties of EPO, focusing on protection against ischemic injury in the heart and its possible future implications for treatment of acute coronary syndromes and post-myocardial infarction (MI) heart failure.

Non-hematopoietic effects of EPO

Originally, EPO was thought to act exclusively on erythroid progenitor cells. This concept was firstly challenged when EPO-R expression was found also in various non-hematopoietic tissues and cells, including neurons⁽¹²⁾, astrocytes, endothelial cells⁽¹³⁾ and vascular smooth muscle cells⁽¹⁴⁾. Binding of EPO to these receptors induces a range of cellular responses that regulate cell survival, proliferation, and differentiation.

Numerous experimental studies elucidating the non-hematopoietic effects of EPO were performed in neural tissue. Both EPO and EPO-R are synthesized in distinct areas of rodent⁽¹⁵⁾ and mammalian brain⁽¹⁶⁾. Hypoxia stimulates the transcription of EPO and EPO-R mRNA in neuronal cultures, as well as in vivo⁽¹⁷⁾. Although only a weak expression of EPO and its receptor has been reported in human brain, ischemic stroke is associated with an acute upregulation of EPO protein- mainly in blood vessels, while EPO-R is amplified in neurons and glial cells in the peri-infarct zone⁽¹⁸⁾.

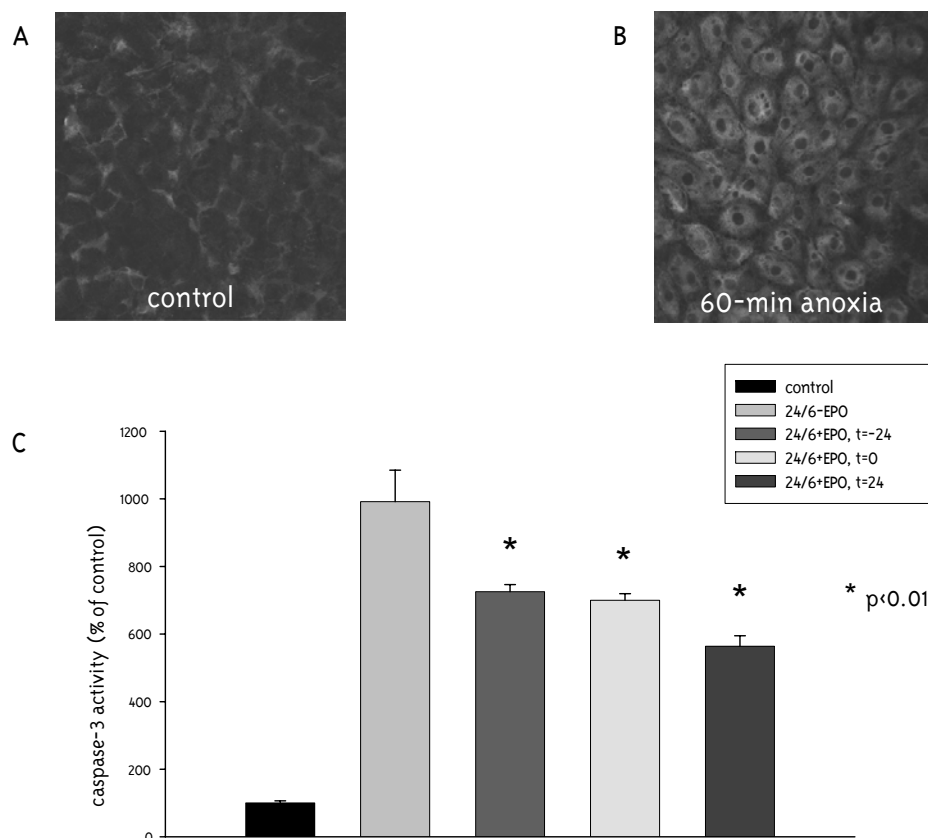
Many experimental studies have shown the protective effect of exogenous EPO treatment in the brain. Systemic administration of EPO before or up to 6 hours after focal brain ischemia reduced the infarct volume by 50-75%⁽¹⁹⁾. Specificity and biological relevance of these changes were demonstrated by the observation that local neutralization of EPO with soluble EPO-R augments ischemic brain damage⁽²⁰⁾. Treatment with EPO is neuroprotective also in animal models of mechanical trauma, toxic injury and neuroinflammation⁽¹⁹⁾.

In most models, inhibition of apoptosis was documented as the underlying mechanism of the observed neuroprotection⁽²¹⁾. This is in correspondence with anti-apoptotic action of EPO on erythroid precursor cells in the bone marrow. Per analogiam, the same intracellular pathways characteristic for EPO signaling in the erythroid precursors (PI3K/Akt, MAPK, STAT-5) are involved also in neurons⁽²²⁾. The activation of these pathways leads to upregulation of anti-apoptotic proteins, such as Bcl-xl, the expression of which was increased after EPO treatment in forebrain ischemia⁽²³⁾. Specific inhibitors of MAPK and PI3K/Akt largely abolished the EPO-induced inhibition of apoptosis⁽²²⁾.

Apart from inhibition of apoptosis, anti-inflammatory and anti-oxidative properties of EPO may contribute to the cytoprotection. However, as for the attenuation of ischemia-induced inflammation by EPO, this may be attributed to the reduction of neuronal death, rather than to a direct effect upon inflammatory cells⁽²⁴⁾. The suppression of nitric oxide mediated free radicals formation has also been suggested to contribute to the neuroprotective effect of EPO⁽²⁵⁾. EPO may also act as a free radical scavenger and directly inhibit lipid peroxidation after ischemia/reperfusion (I/R) induced oxidative damage in the brain⁽²⁶⁾.

The convincing results of the experimental studies led to the first clinical trial with EPO administration in patients with acute stroke⁽²⁷⁾. In this randomized, double-blind trial, rhEPO was given to patients with ischemic stroke presenting within 8 hours after the onset of symptoms. In spite of the relatively small number of patients in this study (n=40), EPO administration in high-doses (entire dose 100.000 IU/ given in three days) proved to be both safe and beneficial. Rather than increasing the hematocrit, EPO treatment merely prevented the decrease in hematocrit observed in the control group. Patients randomized to the EPO group showed significant improvement in clinical outcome parameters and a trend toward smaller infarct sizes, assessed by MRI. Furthermore, the serum levels of brain infarct damage marker (S100 β) were significantly attenuated in EPO-treated group. At present, a multicenter study "EPO in stroke" is being carried out in Germany including patients with moderate to

Figure 1. A, B) Expression of EPO receptor on bovine arterial endothelial cells (BAEC) during normoxia and after 60-min anoxia. C) BAEC were subjected to 24-hour anoxia, followed by 6-hour reoxygenation (24/6). EPO (10 IU/ml) was administered 24 hours before anoxia ($t=-24$), at the start of anoxia ($t=0$) and at the start of reoxygenation ($t=24$) (unpublished data). * $p<0.01$ vs. control.



severe infarcts and a 1-year follow-up (²⁸).

EPO's protective properties were established also in other tissues. In the kidney, treatment with recombinant EPO significantly reduced the renal injury and dysfunction associated with I/R in rodents (²⁹). This effect was related to the prevention of apoptosis by inhibiting the activation of caspase-3, a crucial step in the cascade of programmed cell death (³⁰).

Other reports documented benefit of EPO therapy in the setting of hypoxic retinal disease (³¹), gastrointestinal ischemia (³²), as well as in the cardiovascular system (³³⁻³⁶).

EPO and EPO-R in cardiovascular system

The expression of EPO-R was found in a variety of cell lines originated from cardiovascular system. EPO-R is synthesized and present on the surface of human vascular endothelial cells (¹³). Short-term anoxia of bovine endothelial cells induces up to a two-fold increase in protein levels of EPO-R and exogenous EPO inhibits apoptosis of these cells when administered

at various time points during hypoxia/reoxygenation (unpublished data; figure 1). *In-vitro*, EPO administration prevented apoptosis of endothelial cells subjected to hypoxia, through direct modulation of PI3K/Akt phosphorylation ⁽³⁷⁾. In these cells, EPO was also shown to trigger the phosphorylation of STAT-5 and MAPK ⁽³⁸⁾. Since apoptosis after myocardial infarction begins in endothelial cells and spreads to surrounding myocytes ⁽³⁹⁾, protecting the endothelium may play a crucial role in preserving the myocardial structure.

The expression of EPO-R was demonstrated also in neonatal ⁽⁴⁰⁾ and adult rat cardiac myocytes ⁽⁴¹⁾. Similar to endothelial cells, EPO prevented the apoptosis of cardiomyocytes, by means of activating PI3/Akt pathway.

In-vivo, EPO-R is expressed in normal rat cardiac tissue. Immunostaining for EPO-R is predominantly observed in interstitial cells, including endothelial cells and fibroblasts, with weak expression in cardiomyocytes ⁽⁴²⁾. Perfusion of isolated rat hearts with EPO results in increased MAPK phosphorylation ⁽⁴²⁾, which has been implicated as a survival pathway in cardiac cells during I/R injury, by inhibiting apoptosis ⁽⁴³⁾. Recently, EPO-R expression was confirmed also in human heart tissue. Both ventricular myocytes and endothelial cells in the adult human heart were positive for the EPO-R ⁽⁴⁴⁾.

Expression of EPO itself in the heart, either under normal or hypoxic conditions, has not been reliably established ⁽³³⁾.

An entirely new concept of EPO-mediated protection was introduced by Leist et al. ⁽⁴⁵⁾, through generating mutants of EPO, which do not bind to the classical dimeric EPO-R and lack hematopoietic activity, but nevertheless retain their protective properties in various models of tissue and organ ischemia. This could suggest existence of different types of receptors needed for hematopoietic and non-hematopoietic effects of EPO. Recently, a distinct receptor configuration mediating the tissue-protective EPO effects was identified, consisting of the EPO-receptor and beta-common receptor, which is present also in the myocardium ⁽⁴⁶⁾.

Acute cardioprotective effects of EPO

In hematopoietic and non-hematopoietic tissues alike, EPO was shown to activate pathways leading to inhibition of apoptosis. Apoptosis, one of the major forms of cell death, has been implicated in different cardiovascular diseases. In myocardial infarction (MI) apoptosis might be a determinant of the final infarct size and its extent depends on the presence of post-ischemic reperfusion ⁽⁴⁷⁾.

Recently, numerous *ex vivo* (in isolated rodent hearts) and *in vivo* studies have shown protective role of EPO during ischemic and ischemia/reperfusion (I/R) injury in the heart (Table 1). Pre-treatment of adult rats with EPO (5.000 IU/kg) 24-hours preceding I/R increased the functional recovery of isolated hearts during reperfusion ⁽³³⁾. This was accompanied by protection against apoptosis, as measured by the number of TUNEL positive cells and activity of pro-apoptotic marker caspase-3. The mechanism of EPO mediated protection was revealed by blockade of these effects with a specific inhibitor of PI3K/Akt pathway ⁽⁴⁸⁾. The involvement of signal transducing pathways was further elucidated by Shi et al. in a rabbit isolated heart model, where the cardioprotective effects of EPO were abolished by inhibitors of two different MAPK (p38 and p42/44) ⁽⁴⁹⁾. In the same study, potassium channels inhibitors also blocked the EPO effects, indicating a possible role of potassium channels in EPO-mediated preservation of heart function, possible due to reduction of calcium overload. In a study

Table 1. Experimental studies showing acute cardioprotection with EPO. I/R- ischemia/reperfusion.

	Animal model	Dosage of EPO	Outcome	Source
Ex-vivo I/R	Isolated rat heart- 30 min/2 hours I/R	5000 IU/kg 24 hours before I/R	Increased functional recovery, apoptosis inhibition	33,48
	Isolated rabbit heart- 30 min/35 min I/R	0.5-10 IU/ml, 15 min prior to I/R	Increased recovery, through activation of MAPK and potassium channels	49
	Isolated rat heart- 40 min/2 hours I/R	10 IU/ml throughout the protocol	Improved recovery of left ventricular pressure, coronary flow, reduction of cellular damage	42
	Isolated rat heart- 20 min/25 min I/R	10 IU/ml before I/R	Improved post-ischemic recovery of LVDP, preservation of ATP levels in ischemic myocardium	41
In-vivo I/R	Rat- 30 min/7 days I/R	5000 IU/kg for 7 consecutive days	Reduction in cardiomyocyte loss by 50%, normalization of hemodynamic function	34
	Rabbit- 30 min/ 3 days I/R	5000 IU/kg at the time of reperfusion	Decreased infarct size, enhancement of LV function, mitigation of myocardial cells apoptosis	36,53
	Rat- 40 min/ 24 hours I/R	5000 IU/kg 30 min after ischemia	Reduced infarct size, dependent on MAPK, PI3K/Akt activation	54
	Rat- 40 min/24 hours I/R	5000 IU/kg at different time points during I/R	Reduction in infarct size and apoptosis even when EPO administered after the onset of reperfusion	35
	Dog- 90 min/6 hours I/R	100-1000 IU/kg just before reperfusion	Reduction in infarct size with low-dose, via PI3K dependent pathway	55
In-vivo permanent occlusion	Rabbit- 3 days follow-up	5000 IU/kg at the time of coronary artery ligation	Improvement in inotropic reserve, inhibition of apoptosis	36
	Rat- 8 weeks follow-up	150-3000 IU/kg after coronary occlusion	Smaller infarct size, prevention of LV dilation, improved LV ejection fraction	57,58
	Rat- 9 week follow-up	40 ug/kg darbepoetin after coronary occlusion	Reduction in infarct size, improved LV function, increased capillary density	70

performed by our group, perfusion of isolated rat hearts with EPO during I/R attenuated the deterioration of left ventricular (LV) pressure and coronary flow during the reperfusion (⁴²). EPO in this setting reduced also cellular damage (purine outflow) and number of cells entering apoptosis (active caspase-3 positive). This rapid cardioprotective effect of EPO during I/R injury is also associated with preservation of ATP levels in the ischemic myocardium (⁴¹), linking the anti-apoptotic function to metabolic normalization and consequently functional improvement.

Although inhibition of apoptosis is widely accepted as a mechanism for EPO-mediated protection against acute ischemic injury, other possibilities should also be considered. Pretreatment with EPO was shown to inhibit the I/R induced myocardial inflammatory response (⁵⁰), by preventing the “switching” of myocytes to proinflammatory phenotype, possibly also through upregulation of NO production. EPO can improve the cardiac function also by directly modulating the cardiac Na⁺/K⁺ pump (⁵¹) or stimulating the production of atrial natriuretic peptide in cardiac atrium (⁵²).

In the first *in vivo* study, Calvillo et al. (³⁴), employed a rat model of coronary I/R. Administration of EPO (5.000 IU/kg/day) for seven consecutive days after reperfusion reduced the loss of cardiomyocytes by 50%, an extent sufficient to normalize hemodynamic function. However, the hematocrit increased by 20-30% by the end of the study and such a change alone may lead to improved cardiac function merely by improving the delivery of oxygen.

In a rabbit model of MI, treatment with EPO at the time of permanent coronary ligation resulted in a trend towards reduced infarct size and improvement of cardiac contractility and relaxation when measured 3 days later. Moreover, the cardiac inotropic reserve, studied in response to beta-adrenergic receptor agonist, was significantly enhanced in the EPO-treated group (³⁶). In addition, EPO administration in an *in-vivo* I/R was also found to be beneficial, resulting in a significant reduction of infarct size, expressed as a percentage of total ischemic area at risk (⁵³). This protection was associated with the mitigation of myocardial cell apoptosis. Importantly, no significant differences in hematocrit levels were noted until day 3 after the infarction, discerning the hematopoietic from direct cardioprotective effects of EPO. Furthermore, in line with the results from *ex vivo* experiments, reduction of infarct size *in vivo* is dependent on the activation of pro-survival pathways PI3K/Akt and MAPK (⁵⁴).

The results of these studies raised two clinically important issues: that of the timing and the dosage of EPO administration. In a study performed by our group (³⁵), EPO reduced infarct size and inhibited apoptosis when administered before the actual ischemic episode to until after the onset of reperfusion, providing a broad “window of opportunity” for the potential treatment of acute coronary syndromes. Since most of the previous experimental studies used high doses of EPO, parallel to those demonstrating neuroprotection (1.000-5.000 IU/kg), Hirata et al. (⁵⁵) approached the issue of minimal EPO dose still rendering cardioprotection. EPO treatment in a canine model of I/R reduced infarct size in a dose-dependent manner, establishing the lowest effective dose of 100 IU/kg.

The role of endogenous EPO/EPO-R system was revealed in a study with transgene mutant mice, which express EPO-R exclusively in the hematopoietic lineage (⁵⁶). The infarct size after I/R injury in these mice was larger than in the wild type, subsequently accelerating the development of left ventricular remodeling.

Importantly, the effects of EPO administration persist over a longer period after the ischemic insult. Moon et al. (⁵⁷) found that a single dose of EPO (3.000 IU/kg) immediately after coronary artery ligation in rats reduced the infarct size to 15-25% that of untreated animals,

examined 8 weeks later. This reduction in myocardial damage was accompanied by prevention of LV dilation and improved LV ejection fraction, as measured by repeated echocardiography. Importantly, the single EPO dose did not significantly increase hematocrit. This study was further elaborated by establishing the lowest effective dose (150 IU/kg) and extension of the therapeutic effect with higher doses up to 12 hours after MI⁽⁵⁸⁾. It seems that EPO may protect the myocardium also against more severe insults, such as permanent coronary occlusion and this effect becomes even more pronounced with time. Interestingly, for any given infarct size, EPO treatment has been shown to increase LV ejection fraction over a longer time frame⁽⁵⁹⁾, suggesting benefit on the infarct healing and post-MI remodeling.

Long-term effect of EPO in the heart

Current therapy in patients after MI is focused on prevention of ventricular remodeling and development of heart failure. Myocardial regeneration may offer possibilities that could improve cardiac function in these patients⁽⁶⁰⁾.

Although regeneration of cardiomyocytes by proliferation or transdifferentiation seems limited⁽⁶¹⁾, the formation of new vessels in non-infarcted part may indirectly save cardiomyocytes and lead to improvement of ventricular function.

Two processes contribute to postnatal formation of blood vessels. Angiogenesis is the sprouting of new vessels from existing ones, while vasculogenesis refers to formation of blood vessels from endothelial progenitor cells (EPCs)⁽⁶²⁾. These cells are undifferentiated bone marrow cells, possessing the ability to mature into the cells lining the lumen of a blood vessel. Cytokine-induced mobilization of bone marrow cells after experimental MI leads to smaller infarct size and progressive improvement of ejection fraction, associated with forming of new vascular structures⁽⁶³⁾. After induction of MI, bone marrow-derived EPCs were found in foci of neovascularization at the border of the infarct⁽⁶⁴⁾.

EPCs are mobilized also in patients during acute cardiac ischemic event⁽⁶⁵⁾. Recently, increased levels of circulating EPCs were associated with reduced risk of death from cardiovascular causes in patients with confirmed coronary artery disease⁽⁶⁶⁾. In the BOOST trial, intracoronary infusion of autologous bone marrow cells (CD34+) after myocardial infarction resulted in improved global LV ejection fraction 6 months after cell transfer⁽⁶⁷⁾.

EPO was found to be a potent stimulus for EPCs mobilization, which was associated with neovascularization of ischemic tissue⁽⁶⁸⁾. The effect of EPO on the formation of new vessels has also been observed in an experimental model of stroke. EPO treatment, initiated 24 hours after induction of stroke, enhanced neovascularization and improved neurological function, while it did not significantly influence infarct size⁽⁶⁹⁾. In this study, high-dose EPO treatment for seven consecutive days (5.000-10.000 IU/kg/day), increased the density of microvessels at the stroke boundary (ischemic penumbra), but it also resulted in a 44% increase in hematocrit level.

We addressed this issue in the heart, evaluating the effect of EPO treatment on new vascular formation in an experimental heart failure model⁽⁷⁰⁾. Rats were subjected to coronary artery ligation and therapy with high-dose EPO analogue darbepoetin (40 µg/kg/3 weeks) was initiated 3-weeks post-MI. Although not reducing infarct size, EPO treatment significantly improved cardiac function. This improvement was coupled to increased capillary density and capillary-to-myocyte ratio, indicating neovascularization. Furthermore, these beneficial

effects were also associated with increased percentage of alpha-MHC (myosine-heavy chain) isoforms, a molecular marker of enhanced myocardial contractility.

In the clinical setting, treatment with rEPO causes a significant mobilization and functional activation of EPCs in patients with renal anemia, as well as healthy subjects ⁽⁷¹⁾. EPO treatment in heart failure patients, while not influencing the number, significantly increased the adhesive and proliferative properties of EPCs ⁽⁷²⁾. Interestingly, serum levels of EPO were significantly correlated with the number of circulating EPCs also in patients with established coronary artery disease ⁽⁶⁸⁾.

Besides stimulating and mobilizing EPCs, directly enhancing angiogenesis by EPO could also lead to neovascularization. EPO stimulates proliferation of endothelial cells *in situ* and their differentiation into vascular structures ^(73;74). In human cultured myocardial tissue, EPO stimulated capillary outgrowth comparable to VEGF ⁽⁷⁵⁾. Treatment with EPO ameliorated healing of an ischemic skin wound, an effect attributable to increased microvessel density and higher level of VEGF expression ^(76;77).

However, the doses used in the previous studies, when applied to clinical situation, could cause EPO overdose that may lead to hypertension, seizures, vascular thrombosis and death, possibly related to abruptly increased hematocrit values ⁽⁷⁸⁾. This would be even of a greater concern in patients with already elevated cardiovascular risk.

This considerable clinical problem was addressed by Bahlmann et al. ⁽⁷⁹⁾. In their model of progressive renal disease, treatment with a low-dose EPO analogue darbepoetin conferred tissue protection and preserved capillary network in the kidney, but did not raise hematocrit. Also in patients with renal anemia, standard therapeutic dose of darbepoetin, markedly enhanced the proliferation (i.e. by about 300%) and differentiation of EPCs, compared with one order of magnitude lower increase in the number of red blood cells (i.e. about 30%) ⁽⁸⁰⁾. In a recent study performed by our group we studied the effects of low (non-erythropoietic) dose darbepoetin in a rat model of post-MI heart failure. We showed that darbepoetin treatment preserves cardiac function, even in doses not affecting hematocrit level. This is associated with raised number of circulating EPCs and an increased capillary-to-myocyte ratio (submitted). Stimulation of EPCs, without increasing the number of red blood cells, implies specific EPO dose-effect relationships in different bone marrow cells.

While time-limited treatment with high-dose EPO may be beneficial and safe during acute ischemic injury, if prolonged therapy is required (heart failure), drug regimens using low-dose EPO may be more suitable in avoiding the adverse effects of the treatment. In this regard the non-hematopoietic EPO derivatives could also prove valuable, however their effect on vasculo- and/or angiogenesis is not yet established.

Clinical implications

Erythropoietin has been successfully used in clinical practice for more than two decades, for the most part to treat anemia in patients with chronic kidney disease (CKD), caused by diminished production of endogenous EPO. In these patients, anemia is an established risk factor for cardiovascular (CV) disease outcomes ^(81;82). Numerous smaller studies in pre-dialysis and dialysis patients have demonstrated a beneficial effect of anemia treatment with EPO on various surrogate CV endpoints ^(83;84). However, no conclusive data from randomized controlled trials exist establishing the impact of anemia treatment on cardiovascular and total

mortality in patients with CKD ⁽⁸⁵⁾. A recently started TREAT study will determine the effect of darbepoetin treatment on cardiovascular events in CKD patients ⁽⁸⁵⁾.

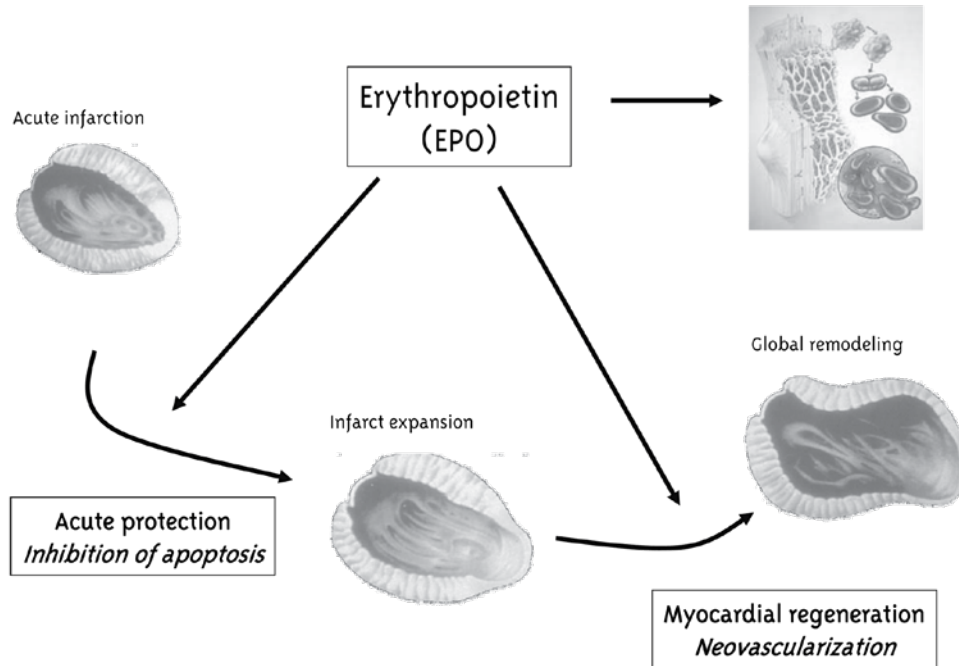
Anemia is also commonly observed in patients with chronic heart failure (CHF) and is related to the severity of the disease ⁽⁸⁶⁾. Although the cause of anemia in these patients is multifactorial, inadequate EPO production and blunted response to EPO in the bone marrow play a major role ⁽⁸⁷⁾. Consequently, elevated EPO levels are associated with the severity of CHF and are a prognostic marker for impaired survival, independent of hemoglobin levels ^(88,89). It seems that, although increased in absolute terms, EPO levels in anemic CHF patients are still relatively low to adequately stimulate the hematopoiesis in bone marrow. Higher levels of hematopoiesis inhibitors, such as Ac-SDKP, could also counterbalance the effects of EPO ⁽⁹⁰⁾. In a number of small studies, normalization of hemoglobin levels with EPO in CHF patients was associated with improved LV ejection fraction ⁽⁹¹⁾ and enhanced exercise capacity ⁽⁹²⁾. While this amelioration could be partly attributed to hemoglobin elevation and thus increased oxygen-binding capacity of blood, the non-hematopoietic effects of EPO must also be considered. Recently, two larger placebo-controlled phase II studies with EPO treatment in anemic CHF patients were conducted which also suggested a potential beneficial effects both in terms of quality of life, and clinical endpoints. For this reason a larger trial aimed at assessing its effect on morbidity and mortality is now being initiated.

The non-hematopoietic tissue-protective properties of EPO may also be beneficial in treatment of patients with acute coronary syndromes. Currently, the emphasis in the treatment of myocardial infarction (MI) is on early reperfusion and hence limiting the damage of cardiac ischemia. However, development of post-MI heart failure remains a major challenge despite “optimal” therapy. EPO could on one hand acutely protect myocardial cells against ischemia/reperfusion induced injury and on the other hand attenuate the cardiac remodeling by stimulating the EPCs. Interestingly, high levels of *endogenous* EPO in patients with first MI who underwent successful primary coronary intervention (PCI), were found to be associated with smaller infarcts, which was interpreted by the authors as a possible endogenous, protective mechanism ⁽⁹³⁾.

We recently performed a first safety and feasibility study with darbepoetin treatment in patients with an acute MI ⁽⁹⁴⁾. In this single-center, investigator-initiated, prospective study we randomized 22 non-anemic patients with a first acute MI to one bolus of 300 µg darbepoetin alfa or no additional medication before PCI. Administration of darbepoetin was both safe and well tolerated. In the darbepoetin group, serum EPO-levels increased to 130-270 times that of controls, within the first 24-hours. Darbepoetin administration led to only small and non-significant changes in hematocrit levels, while endothelial progenitor cells (CD34+/CD45-) were significantly increased at 72-hours. Despite a non-significantly longer “time to treatment” and more extensive baseline area at risk (cumulative ST-elevation) in the darbepoetin-treated group, left ventricular ejection fraction after 4 months was similar in the two groups (52±3% in darbepoetin vs. 48±5% in control group, p=NS).

Recent studies showed that cardiovascular mortality in patients with acute coronary syndromes further increases as hemoglobin levels become lower ^(95,96). Specific therapeutic strategies, including EPO treatment, in such anemic patients with MI should also be further considered.

Figure 2. Biological functions of EPO. "Classical" hematopoietic effect and two mechanisms by which EPO renders cardioprotection in experiment.



Conclusions

A traditionally hematopoietic hormone erythropoietin is increasingly recognized as a pleiotropic cytokine, with effects reaching much further than stimulating red blood cells production⁽⁹⁷⁾. Although already used in cardiology to correct anemia in CHF patients, the non-hematopoietic effects of EPO may be beneficial also in non-anemic cardiovascular patients.

From the experimental studies, EPO appears to influence two crucial processes during cardiac ischemic injury, first by acutely reducing the infarct size and inhibiting the apoptosis, and second by promoting new vessels formation over a longer time frame (figure 2).

In clinics, randomized trials should translate the results of experimental studies and investigate the effectiveness of EPO treatment in various patient populations (acute MI, CHF).

Although treatment with EPO is generally well tolerated and safe, it may be associated with adverse effects, such as hypertension and thromboembolism⁽⁹⁸⁾. These side effects are related mainly to high-dose chronic EPO treatment, associated with increased hematocrit. Using variants of EPO without hematopoietic effect, but retaining tissue protective activity could be useful in a clinical situation, where multiple EPO administrations would be warranted.

Future studies should determine a place for EPO in the treatment of acute and chronic cardiac ischemia, which may have widespread implications for managing heart patients.

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