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Review

Erythropoietin in cardiovascular diseases

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Several studies showed that anaemia is commonly observed in patients with Chronic Heart Failure (CHF) and is associated with worsened symptoms and survival. When anaemia in these patients is treated with erythropoietin (EPO), a significant improvement in cardiac function and symptoms was observed. Although it was originally believed that EPO specifically acted on haematopoietical cells, recent evidence demonstrated several non-haematopoietical effects. Ischaemia/reperfusion experiments in rat heart and brain showed large infarct reduction when treated with EPO. Other effects of EPO are related to its pro-angiogenic effects on endothelial cells, which could be of potential value in patients with ischaemic heart disease. These preclinical findings suggest that EPO may have potential effects in cardiovascular disease beyond correction of haemoglobin levels.
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Introduction

In response to ischaemia, mammalian cells express a variety of proteins, including hypoxia inducible factor 1a (HIF-1a). Expression of HIF-1a increases exponentially, as cellular O_2 concentration decreases.[1] Downstream effects of increasing levels of HIF-1a are upregulation of various proteins of which erythropoietin (EPO) plays a crucial role. EPO acts as a major regulator of erythropoiesis, by promoting the survival and proliferation of erythroid precursor cells.[2] In response to hypoxia the kidney produces EPO, which in turn increases the number of red blood cells and thereby increasing the tissue oxygen supply.

Cloning of the human EPO gene was achieved in 1983. After the first clinical trials with recombinant human erythropoietin (rh-EPO), it has been used for more than a decade in the treatment of anaemia in end stage renal failure.[3] Rh-EPO has a direct effect on haematopoiesis, reflected by increased haemoglobin (Hb) levels. Decreased Hb levels are also common in patients with chronic heart failure (CHF) and although plasma EPO levels are increased in patients with CHF, they are still insufficient to counterbalance the decreased Hb levels.[4] A recent study in anaemic CHF patients showed that rh-EPO therapy significantly improved cardiac function and quality of life after correction of the Hb.[5]

However, EPO can also exert non-erythropoietic effects. Recent evidence suggests that administration rh-EPO plays a protective role in vascular diseases.[6–8] Ischaemia/reperfusion experiments in rat heart and brain showed large infarct reduction when treated with EPO. The favourable effects of these EPO-related changes are still mostly unknown, but may be protection from apoptosis and its antioxidative properties.[9,10] Other benefits of EPO in vascular disease and CHF may be related to its pro-angiogenic potentials.[11] In this review, we discuss the current use of rh-EPO in cardiovascular diseases and its possible novel applications.

Renal failure, anaemia and cardiac disease

Anaemia is frequently observed in patients with chronic renal disease (CRF). When the definition of anaemia, according to the World Health Organisation, is used (haemoglobin <12 g/dl (7.5 mmol/l) for women, <13 g/dl (8.1 mmol/l) for men and postmenopausal women), over

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80% of patients with a creatinine clearance <25 ml/min are anaemic.[12] Renal anaemia is an important risk factor for the development of cardiovascular disease.[13] A study from Foley et al. showed that each 1 g/dl decrease in mean Hb in a renal failure population was independently associated with the presence of left ventricular dilatation and the development of de novo and recurrent cardiac failure.[14] In addition, each 1 g/dl decrease in mean Hb was also independently associated with mortality. Chronic anaemia causes a long-lasting volume overload, which results in ventricular dilatation.[15] Consequently, the length of the sarcomeres increases, leading to a better overlap between myofilaments. Furthermore, the thickness of the left ventricle increases to counterbalance the increased radius (eccentric hypertrophy). Left ventricular hypertrophy is an independent predictor of cardiovascular disease and significantly reduces life expectancy.[16] A study in New Zealand also demonstrated that anaemia together with hypertension and diabetes is one of the strongest independent predictors of left ventricular hypertrophy in patients with CRF.[13]

EPO treatment in the CRF population

Since the first cloning and clinical testing of rh-EPO, fifteen years ago, the use of rh-EPO has become widespread in the treatment of (renal) anaemia. More than a decade ago Wizemann et al. already showed that rh-EPO treatment in dialysis patients with significant coronary artery disease reduces exercise-induced myocardial ischaemia, assessed by (ECG) treadmill test.[17] Furthermore, besides the direct effect on myocardial oxygenation, correction of the anaemia decreases cardiac output and cardiac workload, thus lowering oxygen consumption.[18,19] Besarab et al. studied 1233 haemodialysis patients with clinically evident ischaemic heart disease or CHF.[20] Patients were randomized to a normal haematocrit group (n=618), in which patients received doses of rh-EPO to achieve and maintain a haematocrit of 42%, and a low haematocrit group (n=615), in which patients received doses of rh-EPO sufficient to maintain a haematocrit of 30%. The primary end-point was time to death or a first non-fatal myocardial infarction. After 29 months, there were 183 deaths and 19 non-fatal myocardial infarctions among the normal-haematocrit group (42%), and 150 deaths and 14 non-fatal myocardials were observed among those in the low-haematocrit group (30%). Although patients in the normal-haematocrit group showed a trend towards increased mortality, it is important to note that higher rh-EPO doses or haematocrit values themselves were not associated with increased mortality. In fact, mortality rates decreased with increasing haematocrit values in each group.

A randomized cross-over study conducted in Australia investigated the effects of normalizing Hb in comparison with sub-optimal Hb levels in haemodialysis patients treated with rh-EPO.[21] Patients with higher Hb levels showed a significantly reduced Left Ventricular End-Diastolic Diameter (LVEDD). In addition, the incidence of LVH was significantly lower in patients with high Hb levels, compared to baseline. A Canadian multicenter trial failed to show that normalization of Hb in patients with asymptomatic cardiomyopathy leads to regression of concentric LVH or LV dilatation.[22] Nevertheless, treatment may have prevented additional LV dilatation. To evaluate the possible effect of rh-EPO therapy on the prevention of LVH, Hayashi et al. studied the effects of rh-EPO in pre-dialysis patients. In patients with partially corrected anaemia (Ht 30%) they observed a trend towards a reduction of LVH, whereas in patients with normalised Ht (Ht 40%) this decrease appeared to be statistically significant.[23]

Taken together, in the later phases of cardiac disease in patients with CRF, when severe LVH or LV dilatation has developed, the effects of correction of anaemia seem to be limited. On the other hand, these studies indicate that rh-EPO treatment has at least a favourable effect on the prevention of LVH, although the numbers of patients in each trial are low. An explanation for its limited effects in advanced heart disease could be the long-lasting development of LVH. The accompanied structural abnormalities of the heart, such as interstitial fibrosis[24] can lead to irreversible changes of ventricular structure and therefore may reduce the effects of treatment with rh-EPO.

Anaemia and chronic heart failure

Anaemia is also commonly observed in patients with CHF and is related to the severity of disease. In 142 patients with CHF, mean Hb concentration decreased from 13.7 g/dl (8.6 mmol/l) in mild CHF (New York Heart Association [NYHA] class I) to 10.9 g/dl (6.8 mmol/l) in severe CHF (NYHA IV).[25] Furthermore, it has been shown that anaemia has a prognostic value.[26] Evidence for a relation between anaemia and cardiac morbidity and mortality was provided by Al-Ahmad et al.[27] They retrospectively examined the Studies Of Left Ventricular Dysfunction (SOLVD) database (n=6.635) and found in univariate analysis that a reduced kidney function and lower haematocrit were both a risk factor for all-cause mortality. After adjusting for traditional cardiovascular risk markers they found that a 1% lower haematocrit was associated with a 1.027 higher risk for all-cause mortality. Similar findings were described by McClellan et al.[28] They studied 665 randomly selected patients with the primary diagnosis CHF and also found in this population that both haematocrit and serum creatinine were independently associated with increased risk of death. Further, Horwich et al. studied the relation between anaemia and mortality in a prospective cohort study in over 1000 patients with advanced heart failure (NYHA class III or IV).[26] They conclude that even mild degrees of anaemia, Hb <12.3 g/dl (7.7 mmol/l), are associated with an impaired survival, and with worsened symptoms and functional status (Fig. 1). More evidence that anaemia is associated with in impaired survival was provided by Ezekowitz et al.[29] They studied a large community-based cohort of patients (n=12.065) and found that anaemia is observed in 17% of the patients and an independent

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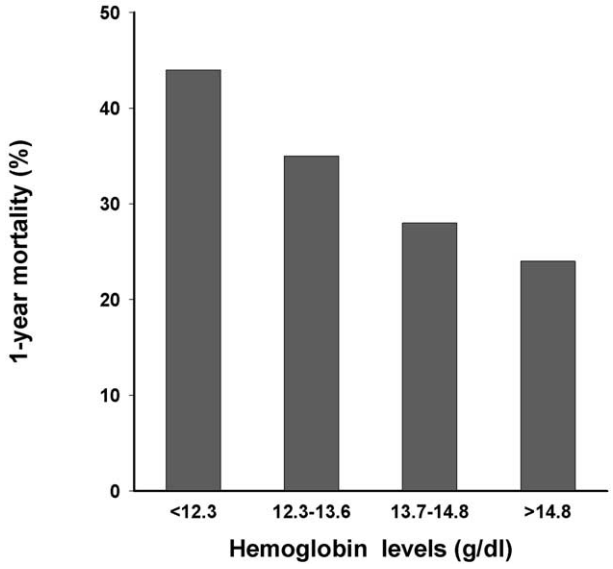

Fig. 1 One-year mortality in NYHA functional class III and IV patients for the different haemoglobin levels.[26]

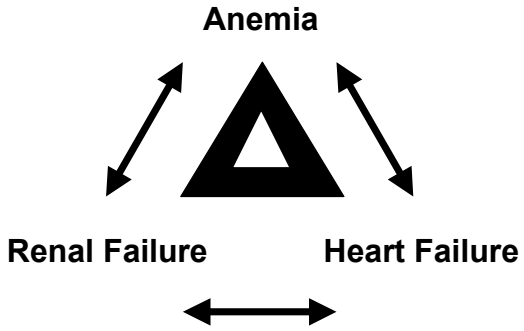

Fig. 2 The cardio-renal anaemia syndrome.[34]

prognostic marker for mortality. To determine the prevalence of anaemia the authors used the International Classification of Diseases. In using this method, they could not provide a cutoff value for when they considered patients to be anaemic. Therefore, this study may underestimate the prevalence of anaemia in community-based patients with CHF.[30]

Several factors play a role in the pathogenesis of anaemia observed in CHF patients. It is known that anaemia in chronic inflammatory diseases is associated with increased levels of cytokines, like Tumor Necrosis Factor (TNF) alpha. Recent studies showed that these cytokines play an important role in heart failure.[31–33] It has been observed that patients with CHF express elevated levels of TNF-alpha, which in turn partly inhibits the haematopoiesis, a mechanism similar to other chronic diseases associated with anaemia. Furthermore, malnutrition could play a role in the development of anaemia in CHF. Horwich et al. showed that CHF patients with lower Hb levels were characterized by lower levels of albumin and a lower body mass index.[26] The use of angiotensin converting enzyme (ACE) inhibitors, widely used in the CHF population, may also reduce the effects of EPO on erythropoiesis[34] and may result in anaemia.[35] In addition, many CHF patients use anti-coagulants, and chronic (microscopic) blood loss may well play a role. Furthermore, anaemia can originate from reduced red blood cell (RBC) volume, but may also result from increased plasma volume (haemodilution). Androne et al. evaluated these two origins of anaemia in patients with CHF and found that haemodilution was observed in 46% of the patients with CHF referred for heart transplantation.[36] In these two cohorts patients with haemodilution tended to have a higher mortality rate than anaemia associated with a reduced RBC volume (P=0.08). These results indicate that treatment should not only be focussed on elevating RBC volume, but also on adjusting the dosage of diuretics in many anaemic patients with severe CHF.

On the other hand, anaemia can also provoke or worsen CHF. The interaction between anaemia and CHF is complex, but there is expanding evidence that CHF can possibly exert anaemia. Silverberg et al. recently proposed the so-called 'Cardio-Renal Anaemia Syndrome', which provides a simple explanation for this phenomenon, where CHF can cause CRF and both can then cause anaemia[34] (Fig. 2). Once anaemia has developed, the increased cardiac workload results in LVH and ultimately worsens the cardiac function, which in turn worsens renal function, completing the vicious circle.

The role of rh-EPO in CHF

A recent study of Silverberg et al. showed the beneficial effects of rh-EPO therapy in CHF patients.[5] They conducted a study in mild anaemic patients with severe CHF (NYHA $\geqslant$III) and treated them with rh-EPO and intravenous iron. Thirty-two patients who had a left ventricular ejection fraction (LVEF) $\leqslant$40% and whose Hb levels were persistently between 10.0 and 11.5 g/dl (6.3–7.2 mmol/l) were randomized into two groups. The treatment group (16 patients) received rh-EPO and intravenous iron to increase the Hb from a mean of 10.3 g/dl to 12.9 g/dl (6.4 to 8.1 mmol/l). Over a mean of 8.2±2.6 months, the treated group showed an improvement of 42% in NYHA class, while NYHA class in the control group worsened with 11%. Furthermore, left ventricular ejection fraction increased 5.5% in the treatment group, compared to a decrease of 5.4% in the control group. The number of hospitalization days decreased by 79% in the treatment group and increased by 57.6% in the control group, compared to the same period before entering the study. Despite the small sample size (n=32) and the open label design of this study, these results strongly suggest an important role for rh-EPO in the correction of even mild anaemia in CHF. Mancini et al. studied the effects of rh-EPO on exercise capacity in patients with moderate to severe CHF.[37] Twenty-six anaemic patients with a mean Hb 11.0 g/dl (6.9 mmol/l) were randomized to receive either EPO (15.000 to 30.000 IU per week) or placebo

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for three months. They found that EPO significantly enhanced exercise capacity, assessed by peak oxygen consumption and increased exercise duration, in patients with CHF. Even more important, quality of life was also significantly improved in the EPO treated group. Recently Silverberg and colleagues conducted a trial to study the effects of EPO treatment in diabetics and non-diabetics with severe CHF and mild to moderate renal failure.[38] They found that correction of mild anaemia (Hb 9.5–11.5 g/dl, [5.9–7.2 mmol/l]) in diabetics and non-diabetics resulted in an improved cardiac function, a better quality of life and a reduction in the number of hospitalizations. Although this was not a randomized placebo-controlled study, the results are comparable with previous studies and add to the expanding literature that diabetics benefit as much as non-diabetics from EPO treatment.

Anti-apoptotic effects of EPO

The previously described beneficial effects of rh-EPO on left ventricular structure and function are mainly explained by its effects on erythropoiesis. However, expanding evidence suggests that EPO plays a major role in non-erythropoietic processes. Several reports showed its efficacy in brain and retinal diseases, mainly by preventing apoptosis.[9,39,40] As apoptosis has been implicated as a mechanism that contributes to the loss of cardiomyocytes in CHF[40] and ischaemic injury,[41] rh-EPO may have beneficial effects on these diseases as well.

Most of the anti-apoptotic effects of EPO are known from the haematological field. EPO is the primary regulator of erythropoiesis, and promotes the proliferation and differentiation of erythroid progenitor cells.[2] It does so, at least partially, by preventing immature erythroblasts from apoptotic cell death. EPO binds to a specific transmembrane receptor: the EPO-receptor (EPO-R). After binding with EPO, various signalling pathways are simultaneously activated, including the MAPK p42/44, JAK2-STAT5 and the PI-3-AKT proteins. Studies performed on immortalized human cell lines suggest that cell proliferation is regulated mainly by activation of MAPK p42/44 or JAK2-STAT5 and inhibition of apoptosis mostly by activation of the PI-3K-AKT axis or JAK2-STAT5.[42] In the latter case, phosphorylated Jak2 triggers the activation of STAT5 protein and the activated STAT5 translocates into the nucleus, where it binds to specific DNA response elements and induces a cascade of cellular responses, including the upregulation of the anti-apoptotic genes such as bcl-2 and bcl-XL.[43,44] This anti-apoptotic mechanism is not only important in erythropoiesis, but also appears to play an important role in other processes with high apoptotic activity, for example in stroke, retinal diseases and possibly myocardial infarction and CHF.[7,40,41,45]

Effects of EPO on angiogenesis

Beside its anti-apoptotic effects, other non-erythropoietic effects of EPO have been described. A study of Juul et al revealed the presence of EPO and EPO-R in human fetal tissue. Although the heart showed minor amounts of EPO, the EPO-R was abundantly expressed in the myocardium as gestation progressed, indicating its presence in adult cardiac tissue.[46] Recently we found that EPO-R is also expressed in the adult heart, including endothelial cells, fibroblasts and to a lesser extent on cardiomyocytes.[47] Experiments with mice deleted (knocked-out) for the gene expressing EPO and EPO-R provided more evidence for its role in cardiac tissue as both $EPO^{-/-}$ and $EPO\text{-}R^{-/-}$ mice suffer from ventricular hypoplasia.[48] This defect appears to be independent from the hypoxic state and is likely due to a reduction in the number of proliferating cardiac myocytes in the ventricular myocardium. To support this concept, additional experiments were performed under cultured conditions. In these experiments, Wu et al. found that EPO acts as a mitogen in isolated cardiomyocytes from $EPO^{-/-}$ and wild type mice, while it has no effect in $EPO\text{-}R^{-/-}$ mice.[48] These findings together strongly suggest that EPO and its receptor, at least during fetal life, stimulate cardiomyocyte proliferation.

Another interesting observation in the experiments with EPO knockout mice was that the vascular network in the mutant rodent was also severely affected, with a disorganized structure. Instead of inter-connected, fine vascular networks, the $EPO\text{-}R^{-/-}$ heart showed dilated and independent vascular clumps.[48] Indirect evidence suggests that subtotally nephrectomized rats with moderate renal failure, and presumably low levels of EPO, showed a lack of microvessels in the heart.[49] The expression of EPO-R has been demonstrated on endothelial cells in vivo and in vitro[50] Stimulation of cultured endothelial cells with rh-EPO resulted in cell proliferation and differentiation into vascular structures[11,51] and incubation of rat aortic rings with rh-EPO was related to endothelial sprouting.[52] Recently, Jaquet et al. compared the angiogenic potentials of EPO with VEGF on endothelial cells derived from the myocardium.[53] They found that both proteins exhibit equal angiogenic potentials, implying a role of rh-EPO in vasoproliferative processes.

Downstream effects of increasing levels of HIF-1α are upregulation of EPO and VEGF, reviewed by Jelkmann et al.[54] It is already known that the latter one causes angiogenesis, which has also been documented in the myocardium. Recent work of our group showed that VEGF might play a role in heart failure, particularly in idiopathic dilated cardiomyopathy (IDC).[55] We observed a decreased capillarization in IDC, which is disproportionate to the rate of hypertrophy and may contribute to the demand-supply mismatch.[56] The reason for this seemingly decreased angiogenic capacity could be the reduced expression of VEGF in IDC. On the other hand, CHF is also associated with an increase in apoptosis.[40,57] Both findings in CHF, decreased capillarization and increased apoptosis could possibly be influenced by rh-EPO due to its anti-apoptotic effects and through stimulation of angiogenesis.

Recently, endothelial progenitor cells (EPCs) have been isolated from adult peripheral blood.[58] It has been shown that they are derived from the bone marrow, and

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play a role in physiological and pathophysiological neovascularization.[58,59] Heeschen et al. provided evidence that EPO stimulates neovascularisation, in part by enhancing EPO mobilization from the bone marrow. In addition, they demonstrated, in a model of hind-limb ischaemia, that EPO increased capillary density by 1.6-fold, compared with control mice. The pathophysiological relevance of these findings was further elucidated in patients with coronary heart disease. They found that patients with unstable coronary heart disease had significantly higher serum EPO levels, which were significantly associated with an increased amount of functionally active EPCs. Therefore, it is tempting to speculate that the dramatic results of EPO treatment in CHF[5] might, in part, be related to the mobilisation of EPCs.

The role of rh-EPO in stroke

One of the first reports on the effects of EPO and its receptor in brain was published in 1995 by Digicaylioglu et al.[60] They detected functional expression of the EPO-R and a hypoxic upregulation of EPO in the brain. Further evidence for this concept was provided by Sakanaka et al.[7] In experiments with gerbils, EPO infusion into the lateral brain ventricle was related to neuronal protection against ischaemia-induced cell death. Specificity and biological relevance of these changes have been demonstrated by their observation that neutralization of endogenous EPO with soluble EPO-R augments ischaemic brain damage. The nature and mechanism of the protective role of EPO was further studied by Siren et al.[9] They linked the prevention of ischaemia-induced cell death to the anti-apoptotic effects of EPO. In rats, focal ischaemia was induced by occlusion of the middle cerebral artery to produce an area of ischaemia surrounded by a penumbra. In the latter area, programmed cell death is prominently present, and the anti-apoptotic effects of systemic rh-EPO therapy are impressive. Administration of EPO after 6 h of arterial occlusion still provided a 50% reduction in infarct size, making rh-EPO a potential candidate for the treatment of ischaemic diseases in humans.

The encouraging findings in animal studies resulted in a double blind randomized proof-of-concept trial to investigate the safety and efficacy of rh-EPO for the treatment of ischaemic stroke in man.[61] Forty patients received either rh-EPO (33.000 U) or saline daily for 3 days after stroke. No adverse events were observed in the EPO treated groups. After one month, they observed an improvement in clinical outcome and a trend towards reduction in infarct size, assessed by MRI-scan, in the EPO treated patients (Fig. 3A). Furthermore, the serum marker of brain infarct damage (S100β) was identical in both groups on admission and increased after time of infarct, peaking at day 7. The rh-EPO group peaked at a lower level than the placebo group (Fig. 3B). However, larger trials are needed to confirm the trends observed in this study.

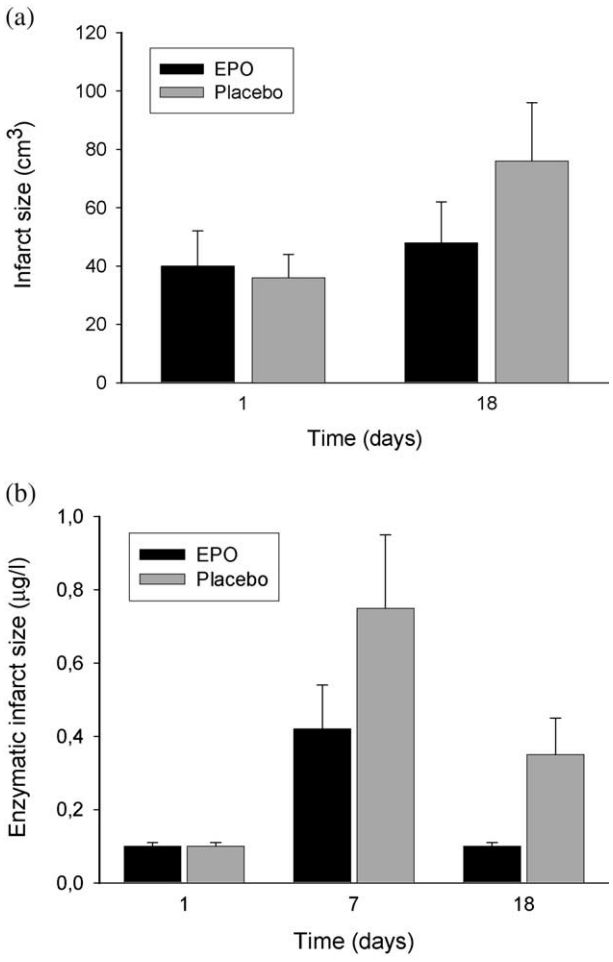

Fig. 3 Evaluation of brain infarct size in patients treated with high dose rh-EPO or placebo for three consecutive days.[16] Data represent mean±SEM. A. Infarct size measured by MRI on baseline and day 18. B. Time course of serum levels of S100, a marker of brain damage.

The role of rh-EPO in ischaemia and myocardial infarction

As there are many similarities between brain and heart ischaemia, recent studies have been conducted to evaluate its possible effect in cardiac ischaemia. Calvillo et al. assessed the potential protective role of EPO in vitro with adult rat cardiomyocytes, and in vivo in a rat model of myocardial infarction with reperfusion.[8] They showed that EPO reduced the amount of apoptotic cells by 30% in cell culture and normalized haemodynamic function within one week after reperfusion in vivo. Recently we conducted ischaemia/reperfusion experiments in the isolated rat heart to evaluate possible beneficial effects of EPO treatment (submitted). Administration of EPO reduced the cellular damage by 56% ($P<0.05$) and apoptosis by 15% ($P<0.05$) during reperfusion and resulted in a significantly improved recovery of left ventricular pressure ($P=0.02$) and coronary flow ($P=0.01$). Although the mechanism through which EPO preserves cardiac function has yet to be elucidated, it is tempting to speculate that anti-apoptotic properties of EPO play a pivotal role.

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A recent paper from Scarabelli et al. showed that in the early stages of reperfusion, apoptosis is first seen in endothelial cells and then spreads to surrounding cardiomyocytes, suggesting reperfusion induced release of pro-apoptotic mediators from endothelial cells.[62] Although ischaemia is able to initiate the apoptotic cascade, reperfusion is required to complete the apoptotic program.[63] This is consistent with the findings that EPO limits cellular damage mainly during reperfusion, and to a lesser extent in the ischaemic period. As the EPO-R in the heart is also expressed on endothelial cells, EPO treatment may prevent apoptosis in endothelial cells during reperfusion and thereby protect the myocardium and preserving vascular flow. Although these studies were all performed in rodents, apoptosis also plays an important role in patients with acute myocardial infarction. Saraste et al. observed apoptotic cardiomyocytes, particularly in the border zones of the infarcted myocardium, comparable to the penumbra in brain ischaemia.[41] As the apoptotic activity reaches its peak during the reperfusion period, beneficial effects of rh-EPO could possibly be achieved in patients receiving trombolytic therapy or after primary Percutaneous Coronary Intervention (PCI). Furthermore, it has been shown that EPO influences, at least during fetal life, cardiomyocyte proliferation. Therefore, it is interesting to speculate that infarct size, on one hand, could be limited through the anti-apoptotic effects of rh-EPO, but also could be influenced by increased proliferation.

Conclusion

Erythropoietin has been used in cardiovascular medicine for many years. Initially, research focussed on the direct effect on haematopoiesis and correction of anaemia. Normalization of the Hb level in mild anaemic patients with CHF showed a positive effect on left ventricular function, a reduction in hospitalization days and even more important an increase in quality of life. Although it was originally believed that EPO specifically acted on haematopoietical cells, recent evidence demonstrated several non-haematopoietical effects. The large reduction of infarction size after rh-EPO therapy in ischaemia/reperfusion experiments in rat heart and brain are promising. As the EPO-R is present in cardiomyocytes and endothelial cells, one could speculate about its effects in other cardiac diseases. The anti-apoptotic effects might be specifically interesting in patients with CHF and myocardial infarction. The pro-angiogenic effects of EPO, as effective as VEGF, are also of potential value in patients with ischaemic heart disease. Taken together, the future of rh-EPO therapy in cardiovascular diseases seems promising. Further basic research into the molecular mechanisms of the apoptosis cascade in cardiomyocytes and pro-angiogenic effects on EPCs and endothelial cells will be necessary to delineate the benefits of rh-EPO therapy in cardiovascular medicine. Finally, randomized clinical trials have to prove if its theoretical advantages will be translated into clinical practice.

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