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Therapeutic Potential of Erythropoietin in Cardiovascular Disease: Erythropoiesis and Beyond

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Erythropoietin (EPO) is a glycoprotein hormone implicated in the regulation of red blood cell production. Anemia is common in chronic heart failure (CHF) patients and associated with an inappropriately low EPO-production, suggesting a role for its recombinant human form (rhEPO) in treatment. Although safety concerns have been raised regarding treatment with rhEPO in patients with chronic kidney disease, treatment with rhEPO in patients with CHF has so far been safe and well tolerated. The effect of rhEPO on outcome in anemic CHF patients is under investigation in a phase III clinical trial. In addition to its erythropoietic effects, EPO has been detected in the cardiovascular system, fueling intense research into possible non-hematopoietic effects. EPO has been shown to exert protective effects on the heart during acute myocardial ischemia and improve cardiac function in experimental CHF. Acute protection is mediated through reduction of apoptotic cell death. Improvement of cardiac function in CHF is related to myocardial neovascularization. EPO exhibits a vast array of beneficial effects in cardiovascular disease. In addition to the correction of anemia in CHF, rhEPO might benefit patients with cardiovascular disease.

Introduction

Erythropoietin (EPO) is a hematopoietic hormone, and its recombinant human form (rhEPO) has provided a breakthrough in the treatment of anemia caused by EPO deficiency in chronic kidney disease. Over the past decade the indication for rhEPO has markedly broadened and now also includes anemia in patients with cancer who are receiving chemotherapy, patients with HIV who are treated with zidovudine, and treatment of myelodysplastic syndromes. rhEPO treatment is also granted before major surgery as a prophylaxis to reduce blood transfusions or for patients who are unwilling to receive blood. Moreover, treatment of anemia with rhEPO in patients with chronic heart failure (CHF) seems safe and well tolerated, leading to a phase III clinical trial currently enrolling patients. Recently, however, a functional EPO receptor (EPOR) was detected outside the hematopoietic system, fueling intense research into possible non-hematopoietic effects. From this, EPO has emerged as a myocardial survival and vascular growth factor with a promising protective potential in the setting of acute and chronic myocardial ischemia. This review focuses on the cardiovascular EPO-EPOR system and the potential role of treatment with rhEPO in cardiovascular patients with or without anemia.

Erythropoietic Effects of EPO

EPO is a glycoprotein hormone mainly implicated in the regulation of red blood cell production. EPO is produced in the fetal liver and the adult kidney under the transcriptional control of hypoxia inducible factor-1α (HIF-1α) [1]. Although EPO is critical for the regulation of erythropoiesis, the production of EPO is not stimulated by diminished red blood cell numbers but instead through the downstream effect on tissue oxygen supply. Under normoxic conditions, the continuous production of HIF-1α is antagonized by immediate degradation in the proteasome, making it functionally inactive. Hypoxia directly inhibits proteasomal degradation, resulting in exponentially increased expression of HIF-1α and transcription of a variety of hypoxia responsive genes including EPO. The renal medulla is especially sensitive to alterations in partial oxygen tension, which together with the ability to

rapidly increase EPO levels and exponentially augment red blood cell production, allow for tight regulation hemoglobin (Hb) levels and consequent tissue oxygen supply.

EPO targets a transmembrane member of the cytokine type 1 receptor superfamily, ubiquitously expressed on erythroid progenitor cells. Binding of EPO results in dimerization of EPOR, which in turn activates the receptor associated janus tyrosine kinase-2 (JAK2). JAK2 further propagates the signal by engaging secondary signal transduction molecules, including transducers and activators of transcription (STAT), mitogen-activated protein kinases (MAPK), and phosphatidylinosol 3-kinase (PI3K)-protein kinase B pathways. The principal effect of EPO is the reduction of physiologic apoptosis associated with cell turnover in erythroid progenitor cells, but in conjunction with other growth factors, EPO additionally stimulates proliferation and differentiation of these cells [2].

Erythropoiesis in CHF

Anemia (defined by the World Health Organization as Hb levels < 13 g/dL in men and <12 g/dL in women) is commonly observed in patients with CHF [3•,4]. Anemia causes chronic volume overload to the left ventricle which results in increased oxygen consumption, left ventricular (LV) dilation, and LV hypertrophy, thereby negatively affecting cardiac function in CHF. Indeed, the presence of anemia in CHF has been consistently associated with impaired survival.

The exact etiology of anemia in CHF has not been fully resolved and is likely to be multifactorial within the population as well as within each patient [3•,4]. In contrast to patients with chronic kidney disease, circulating EPO levels are elevated in patients with CHF, increase with the progression of disease, and independently predict impaired survival [5•]. However, when EPO levels are corrected for the prevailing Hb by calculating the observed/predicted (O/P) EPO ratio, the vast majority of patients with CHF display signs for insufficient EPO production. Opasich et al. [6••] demonstrated that more than 90% of anemic CHF patients display significantly depleted O/P EPO ratios compared with healthy controls, which was recently confirmed by our group [7••]. The impaired EPO production is caused by a combination of decreased renal function and a direct inhibition of EPO production in the kidney by pro-inflammatory cytokines and angiotensin-converting enzyme inhibitors [4]. On the other hand, the relatively elevated EPO levels in CHF are indicative of reduced responsiveness of erythropoietic cells to EPO. Experimental evidence for reduced erythropoiesis in CHF was reported by Iversen et al. [8]. In mice with heart failure after myocardial infarction (MI), the erythropoietic progenitor pool was reduced by 40%, apoptosis was increased and proliferation of these cells markedly impaired. This was associated with increased tumor necrosis factor (TNF)-α/Fas expression and increased cytolytic activity of bone marrow natural killer cells, suggesting an important role for inflammation in erythropoiesis inhibition. Interleukin-1, TNF-α and interferon α, β, and γ directly inhibit the formation of mature erythropoietic cells from erythropoietic progenitors in the bone marrow [9]. CHF is frequently associated with elevated levels of pro-inflammatory cytokines, and markers for inflammation are independently related to elevated EPO levels [7••,10]. Moreover, we have recently demonstrated that anemia in CHF is partially explained by elevated levels of AcSDKP, a negative regulator of hematopoietic stem cells [11•]. The inhibitory effects of inflammatory cytokines and AcSDKP might increase the EPO levels required to maintain adequate red blood cell production. In addition to inhibition by circulating factors, erythropoietic progenitor cells of patients with CHF might exhibit impaired function. Hence, CHF is not primarily associated with defective EPO production but rather an inability to adjust production to an increased demand.

Additional mechanisms may contribute to the development of anemia in CHF. We recently demonstrated that in addition to blunted EPO production and impaired renal perfusion, anemia in CHF is associated with enhanced fluid retention [7••]. Fluid retention can result in expansion of plasma volume and consequent hemodilution which may cause pseudo-anemia. Furthermore, CHF is infrequently associated with biochemical indices of impaired iron supply, but iron stores in the bone marrow are significantly depleted [12••]. Although this might indicate systemic iron deficiency, it is more likely caused by diversion of iron to the reticuloendothelial system as part of the anemia of chronic disease [13]. Finally, the vast majority of patients with CHF use platelet aggregation inhibitors or anticoagulants, which might cause chronic microscopic blood loss. The extent to which this plays a role in CHF is not well described.

The Discovery of a Cardiovascular EPO-EPOR System

The first evidence for a biologic role for EPO outside the hematopoietic system came from tissue expression studies in 1992 which demonstrated EPO messenger RNA in the brain [14]. In addition to the adult kidney and the fetal liver, expression of EPO and EPOR has now been detected in the brain, heart, reproductive organs, and endothelial and vascular smooth muscle cells [15•]. In contrast to the ubiquitous high expression of erythroid cells, expression of EPOR in non-hematopoietic tissues is relatively low [16]. Expression of both EPO and EPOR, however, rapidly increase following hypoxia and a number of other metabolic stressors including pro-inflammatory cytokines, hypoglycaemia, and increased reactive oxygen species. In addition, EPOR expression is stimulated by rhEPO, most notably in conjunction with hypoxia [17]. Hence, although healthy non-hematopoietic tissues are

relatively insensitive to EPO, an ischemic insult will result in autocrine/paracrine production of EPO and increased sensitivity of target cells.

Research into the endogenous significance of this extra-hematopoietic EPO-EPOR system has been hampered by the pivotal importance of its hematopoietic counterpart. EPO and EPOR knockout mice die at 14 weeks gestation due to severe anemia and, in addition, exhibit ventricular hypoplasia and a markedly impaired vascular development [18]. These findings have been interpreted as proof that (cardio)vascular EPOR is crucial for the development of the cardiovascular system, but recent evidence has abrogated this hypothesis [19]. Suzuki et al. [19] developed a murine model in which EPOR expression is restricted to the erythropoietic lineage, by targeted knock-in of the EPOR gene ligated to the GATA-1 promoter, a transcription factor exclusive to erythroid lineage cells. This results in mice that exclusively express the EPOR in erythroid lineage cells while the other organs are devoid of an EPOR. Surprisingly, despite the absence of an EPOR in the cardiovascular system, these mice develop normally and are fertile, indicating that the cardiovascular EPO-EPOR system is dispensable for normal development [19]. Nevertheless, four outstanding publications by the same group revealed that these mice exhibit markedly increased susceptibility to acute and chronic cardiovascular disease, including more extensive MIs after ischemia reperfusion injury [20••], an impaired angiogenic response to femoral artery occlusion [21••], augmented pressure overload–induced LV dysfunction [22••], and accelerated hypoxia induced pulmonary hypertension [23]. The same group demonstrated a correlation between high serum EPO and smaller myocardial infarct size in patients, interpreted as proof of a possible endogenous protective mechanism [24]. The latter findings should, however, be interpreted with caution, as higher EPO levels were associated with lower Hb levels, and the appropriate multivariable corrections were not made. More compelling evidence was recently published by Ferrario et al. [25•], who demonstrated an Hb-independent increase in EPO production after MI, persisting until 7 days after MI. It is unknown whether the increased circulating EPO levels result from cardiac EPO production, although increased EPO-production after MI has been demonstrated in the murine heart. Together these results indicate the existence of an endogenous EPO-EPOR system as part of an endogenous defense mechanism against a broad spectrum of acute and chronic cardiovascular disease.

EPO Treatment for Acute Myocardial Ischemia

The anti-apoptotic effects of EPO are crucial for the regulation of red blood cell production but might become equally important for the treatment of acute myocardial ischemia. Following an acute MI, extensive myocardial apoptosis ensues which in part determines the extent of the permanent myocardial damage. EPO exerts potent anti-apoptotic effects in a number of cellular systems including cultured endothelial cells and neonatal rat cardiomyocytes [15•]. Moreover, EPO prevents apoptosis from a number of sources, but hypoxia is most extensively studied. The signal transduction pathways associated with the anti-apoptotic actions of EPO in extra-hematopoietic cells display remarkable similarities to erythroid cells including PI3K-AKT, STAT, and MAPK. However, research has in part been restricted to these pathways [23]. It is widely accepted that cytoprotection by EPO is caused by its anti-apoptotic effects. Nevertheless, EPO has been linked to an attenuated inflammatory response of cardiomyocytes, associated with upregulation of endothelial nitric oxide synthase (eNOS) [26]. Interestingly, the anti-apoptotic effects of EPO are abrogated by specific eNOS blockers or in cells derived from eNOS knockout mice, suggesting that eNOS upregulation is crucial for the anti-apoptotic effects of EPO as well [27]. The activation of the eNOS pathway might suggest that in addition to the anti-apoptotic effects, systemic EPO treatment might improve endothelial function and consequently reduce peripheral resistance.

Numerous studies have translated the cytoprotective in vitro effects into ex and in vivo models of acute myocardial ischemia reperfusion injury and permanent coronary artery ligation [23]. In the first in vivo study, Calvillo et al. [28] administered a high dose of EPO (5000 U/kg) for 7 consecutive days after myocardial ischemia reperfusion injury in rats, which resulted in a 50% reduced loss of cardiomyocytes and significantly preserved cardiac function, but to a 30% increased hematocrit as well. Although this study was unable to fully separate cardioprotective effects from the hematopoietic effects, other studies have proven the hematocrit independent nature of cardioprotection [29•]. EPO exerts protective effects in models of ischemia/reperfusion injury and permanent coronary artery ligation in mice, rat, rabbits and dogs, with doses ranging from 8000 U/kg to 100 U/kg [29•]. Moreover, protection is induced when EPO is administered before ischemia, during ischemia, and at the onset of reperfusion, providing a broad window opportunity [30]. Finally, the effects of a single dose of EPO on infarct size and cardiac function are still present 9 weeks later [31••]. Together these experimental data suggest a promising role for rhEPO as a cardioprotective agent in the setting of an acute MI.

EPO Treatment in CHF

Irrespective of epicardial coronary anatomy, perfusion of the myocardium is impaired in heart failure due to disproportionate cardiac hyperthrophy relative to (micro)vascular growth, resulting in low-grade ischemia [32,33]. In addition to the negative inotropic effects of impaired perfusion, ischemia will result in a switch from fast energy consuming

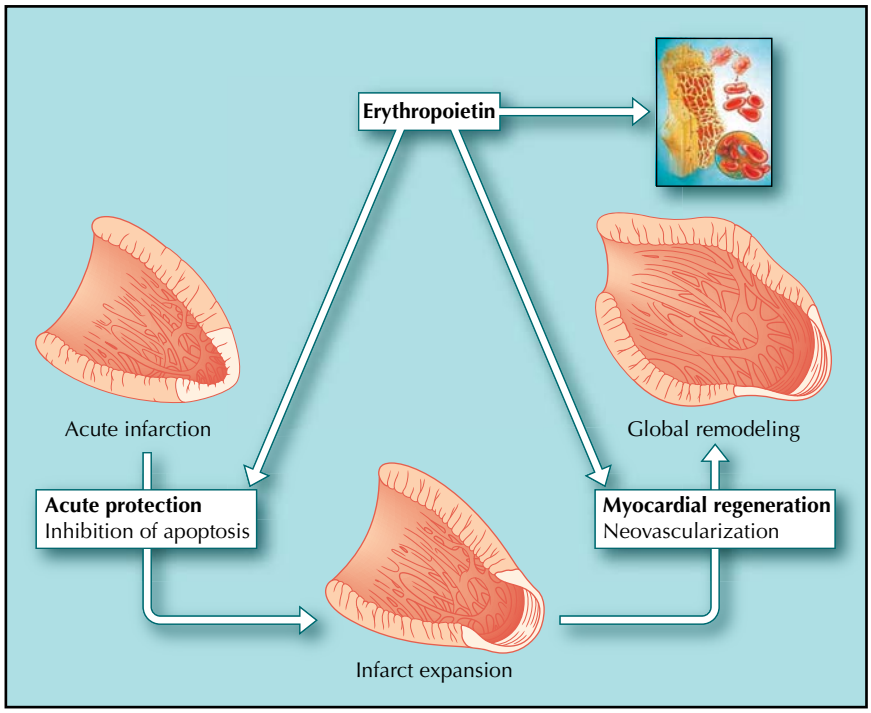

Figure 1. Biologic functions of erythropoietin after myocardial injury—erythropoiesis and beyond.

α-myocin heavy chain (MHC) isoforms to slow β-MHC isoforms, which further impairs contractility.

Therapies aimed at improving cardiac microvascularization might improve cardiac function in CHF. EPO has been shown to stimulate neovascularization by promoting proliferation and survival of endothelial cells in vitro and stimulating angiogenesis in vivo [34–36]. In addition, EPO induces the proliferation, differentiation, and adhesion of a subset of bone marrow derived progenitor cells with an endothelial phenotype (endothelial progenitor cells [EPC]) in vitro and results in marked mobilization of EPC in vivo [37••,38,39]. EPC specifically home to sites of neovascularization and contribute to the formation of new vessels [40]. In order to evaluate the effects of EPO on cardiac function and neovascularization, we induced heart failure in rats by coronary artery ligation and treated them with a high dose (40 μg/kg/3 weeks) of the long-acting EPO analogue darbepoetin alfa, starting 3 weeks after MI [31••]. Although this delayed treatment did not result in a reduction of infarct size measured after 9 weeks of treatment, cardiac function was significantly improved. The improved cardiac function was associated with increased capillary density and increased capillary: myocyte ratio, indicating neovascularization.

The beneficial effects of EPO on cardiac function and microvascularization in post-MI LV dysfunction have recently been confirmed by three independent studies [41–43]. Furthermore, in a distinct model of chronic myocardial dysfunction, EPO prevented doxorubicin induced deterioration of cardiac function which was also associated with neovascularization of the myocardium [44,45]. The EPO-induced neovascularization is consistently associated with increased circulating EPC. Interestingly, in a model of doxorubicin induced myocardial dysfunction, infusion of isolated EPC resulted in improved cardiac function and neovascularization in a magnitude equal to EPO [44]. We recently investigated the contribution of EPC to EPO induced neovascularization by replacing bone marrow of rats with genetically labeled cells, which allows differentiation between EPC mediated neovascularization and in situ proliferation of endothelial cells [46••]. This study revealed that approximately 30% of the new vessels were comprised of bone marrow–derived cells indicating that EPC contribute to EPO-induced neovascularization. The remaining vessels however comprised of in situ proliferated myocardial endothelial cells, associated with a fivefold increased expression of vascular endothelial growth factor (VEGF). EPO-induced neovascularization in post-MI heart failure therefore seems mediated through a combination of EPC recruitment from the bone marrow and increased myocardial expression of VEGF.

The dosing regimens used in previous studies all resulted in a significant increase in hematocrit levels. When applied to the clinical situation, this could lead to hypertension, seizures, vascular thrombosis, and death, possibly related to abruptly increased hematocrit levels [47]. Therefore, we recently compared the effects of a high EPO dose with a low dose that had no effect on hematocrit. Similar to high-dose EPO, low-dose treatment resulted in slightly less pronounced but statistically significantly improved cardiac function and improved myocardial microvascularization (unpublished data). Another option to avoid the potentially negative effects of chronic EPO therapy on hematocrit values could be the use of recently discovered non-erythropoietic derivatives of EPO, retaining the tissue protective property, without the undesired effect

on erythropoiesis [48••]. The possibility to separate the erythropoietic and tissue-protective effects is explained through structural differences between receptors in bone marrow and in "peripheral" tissues [49]. Two independent studies have demonstrated that these non-erythropoietic EPOs retain their cardioprotective potential in models of acute MI [50,51]. It is uncertain whether these new EPOs will improve cardiac function in CHF.

Clinical Perspectives

The observation that anemia in CHF is associated with defective EPO production has prompted the evaluation of rhEPO for its treatment. Several safety and efficacy studies have evaluated correction of anemia in CHF with rhEPO with or without concomitant administration of intravenous iron. The first study was performed by Silverberg et al. [52] who randomized 32 patients with mild anemia to receive either rhEPO and intravenous iron or no additional treatment, resulting in significant improvement in New York Heart Association functional class and cardiac function. These findings have been corroborated by several randomized, double-blind, placebo-controlled studies. In addition to the amelioration of anemia, EPO treatment improved LV ejection fraction, renal function, exercise capacity and quality-of-life scores and reduced natriuretic peptides and hospital admissions for CHF [3•]. A pooled analysis of the two largest studies, which combined the data of more than 320 patients, revealed a trend to reduced risk of the combined endpoint of CHF-related hospitalization and all-cause mortality in the rhEPO-treated groups [53]. Based on these findings, a large multicenter, double-blind, randomized, placebo-controlled trial was designed, which has recently started enrolling patients (RED-HF). On the other hand, the recently published CHOIR study in patients with chronic kidney disease, demonstrated that normalization of Hb values to reference ranges (Hb > 13.5 g/dL) with rhEPO resulted in significantly more cardiovascular events compared to target levels to 11.3 g/dL, confirming previous findings from Besarab et al. [47,54]. These results have intensified the debate on the safety of rhEPO. As a result, the Food and Drug Administration has recently recommended "the lowest possible dose to slowly raise the hemoglobin concentration to the lowest level that will avoid the need for a blood transfusion." Of note, the possible beneficial effects of rhEPO in chronic kidney disease patients seem to be distinctly different from patients with CHF. So far, no deleterious effects of rhEPO have been observed in patients with CHF, but current trials will be carefully monitored.

Besides clinical CHF trials, two phase II trials are running in patients with an acute MI. In this setting, only one bolus of EPO is used, and therefore the risks of an unwanted hematocrit-elevation are very limited. We recently performed a randomized safety and feasibility study with a single 300 μg bolus of the long acting EPO analogue darbepoetin alfa, administered during primary percutaneous coronary intervention for a first acute MI. EPO treatment was both safe and well tolerated, caused only a small but nonsignificant increase in hematocrit levels and significantly increased circulating EPCs [55••]. These findings led to the design of a randomized multicenter study that evaluates whether EPO can attenuate post-MI loss of cardiac function, currently enrolling patients at our center (NCT00449488). The similar REVEAL study is currently performed in the United States (NCT00378352). The results of these studies are clearly awaited.

Conclusions

Although recently scrutinized for other indications, no deleterious effects of rhEPO have been observed in anemic patients with CHF. The efficacy of rhEPO for the correction anemia in CHF is currently under investigation. However, recent evidence has transformed EPO from a designated erythropoietic growth factor to a cytokine that is now recognized for its pleiotropic tissue protective properties. The endogenous EPO-EPOR system is crucial for protection against acute and chronic myocardial ischemia and systemic administration of rhEPO has promising beneficial effects in acute and chronic cardiac disease. Whereas the setting of an acute MI might benefit most from the EPO-induced protection against apoptotic cell death, the chronically failing heart seems to improve through EPO-induced neovascularization (Fig. 1). Therefore, in addition to the correction of anemia in CHF, rhEPO might benefit patients with cardiovascular disease through additional hematocrit independent mechanisms.

Clinical Trial Acronyms

CHOIR—Correction of Hemoglobin and Outcomes in Renal Insufficiency; RED-HF—Reduction of Events with Darbepoetin alfa in Heart Failure; REVEAL—Reduction of Infarct Expansion and Ventricular Remodeling with Erythropoietin after Large Myocardial Infarction.

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