

Erythropoietin in Heart Failure and Other Cardiovascular Diseases: Hematopoietic and Pleiotropic Effects

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Abstract: Erythropoietin is a hypoxia-induced hormone that is a major regulator of normal erythropoiesis. Over the last decade, the production of recombinant human erythropoietin has revolutionized the treatment of anemia associated with chronic renal failure, and has led to a greater understanding of anemia pathophysiology and to the elucidation of the interactions of erythropoietin, iron, and erythropoiesis. Anemia has been shown to be independently associated with increased mortality and disease progression. Potential survival benefits associated with correction of anemia have expanded considerably the indications of erythropoietin use in various patient populations and are leading to consideration of earlier, more aggressive treatment of mild to moderate anemia. The results of such treatment are promising in a variety of new clinical settings, including anemia associated with congestive heart failure. Furthermore, the erythropoietin receptor is widely distributed in the cardiovascular system, including endothelial cells, smooth muscle cells and cardiomyocytes and preclinical studies have established erythropoietin to be a pleiotropic cytokine with anti-apoptotic activity and tissue-protective actions in the cardiovascular system, beyond correction of hemoglobin levels. Despite some potential adverse effects, such as hypertension, and the occurrence of erythropoietin resistance, early studies in heart failure patients with anemia suggest that erythropoietin therapy is safe and effective in reducing left ventricular hypertrophy, enhancing exercise performance and increasing ejection fraction.

Anemia is found in about one-third of all cases of congestive heart failure (CHF). The most likely common cause is chronic renal insufficiency, which is present in about half of all CHF cases. However, anemia can occur in CHF without renal insufficiency and is likely to be due to excessive cytokine production. The anemia itself can worsen cardiac function, both because it causes cardiac stress through tachycardia and increased stroke volume, and because it can cause a reduced renal blood flow and fluid retention, adding further stress to the heart. Long-standing anemia of any cause can cause left ventricular hypertrophy, which can lead to cardiac cell death through apoptosis and worsen CHF. Therefore, a vicious circle, cardio-renal anemia syndrome, is set up wherein CHF causes anemia, and the anemia causes more CHF and both damage the kidneys worsening the anemia and the CHF further and increasing mortality. There is now evidence that early correction of the CHF anemia with subcutaneous erythropoietin and intravenous iron improves shortness of breath and fatigue, cardiac function, renal function and exercise capacity, reducing the need for hospitalization and improving quality of life.

In the present review we discuss the data on current clinical use of erythropoietin in cardiovascular disease, with the main focus on the treatment of congestive heart failure, and summarize the advances and progress made in the understanding of the hematopoietic and pleiotropic effects of erythropoietin in the cardiovascular system.

Key Words: Erythropoietin, anemia, heart failure, cardiovascular disease, myoprotection.

INTRODUCTION

Anemia is a well-recognized risk factor in a variety of medical conditions, including end-stage renal failure [1], but its role in congestive heart failure (CHF) has only recently received attention [2-8]. Up to 30%-50% of patients with CHF have been reported to be afflicted by anemia and in the majority it had been referred to as anemia of chronic disease. From recent studies it appears that the presence of anemia in CHF has an adverse prognosis as an independent prognostic factor of increased mortality [2-8], while its correction has been advocated to improve prognosis in these patients [2,5,8]. Use of erythropoietin (EPO) to correct anemia has a long track record in the management of renal failure [9],

while it has only recently begun being explored in CHF patients [8]. A most common definition of anemia comprises a hemoglobin cut-off level of <12 g/dl. Of course, treatable causes, such as iron, folate, or B₁₂ deficiency should be sought and corrected before considering use of erythropoietin. With regards to other causes of anemia in CHF, several mechanisms have been proposed, such as bone marrow suppression or induction of erythropoietin insensitivity by cytokines (e.g. tumor necrosis factor), which can also interfere with iron release and utilization. Down regulation of EPO by angiotensin converting enzyme (ACE) inhibitors, and/or a relative deficiency of EPO production due to associated functional renal failure have also been considered. Regardless of mechanism, the prospect of correcting the anemia with EPO administration with potential reversibility of increased risk associated with anemia in CHF is a target worth exploring.

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Erythropoietin can also exert non-hematopoietic or pleiotropic effects [10-15]. Recent evidence suggests that administration of recombinant erythropoietin plays a protective role in vascular diseases. Preclinical studies have established erythropoietin to be a pleiotropic cytokine with anti-apoptotic activity and tissue-protective actions in the cardiovascular system [10,11,16,17]. Ischemia/reperfusion experiments in rat heart and brain showed large infarct reduction when treated with erythropoietin. Other effects of erythropoietin are related to its pro-angiogenic effects on endothelial cells, which could be of potential value in patients with ischemic heart disease. These preclinical findings suggest that erythropoietin may have potential effects in cardiovascular disease beyond correction of hemoglobin levels.

In this review we discuss the data on current clinical use of erythropoietin in cardiovascular disease, with the main focus on the treatment of congestive heart failure, and summarize the advances and progress made in the understanding of the hematopoietic and pleiotropic effects of erythropoietin in the cardiovascular system, as a member of the large and diverse cytokine superfamily.

RECOMBINANT HUMAN AND OTHER FORMS OF ERYTHROPOIETIN

Human erythropoietin (EPO) is an endogenous 30.4 kDa glycoprotein hormone containing a 165-aminoacid residue backbone and two disulphide bonds (Fig. 1), which regulates red blood cell production (erythropoiesis) [18,19]. EPO is induced by hypoxia and it is synthesized by peritubular cells in the cortex-medullary border of the kidney and in the liver during fetal and neonatal development. It has similar structure and signaling mechanisms to the family of type I cytokines. The purification of erythropoietin from the urine of patients with aplastic anemia, reported in 1977, permitted cloning of its gene [20,21]. The recombinant therapeutic agent was first synthesized in 1985, only 2 years after the EPO gene was cloned, and was approved for clinical use in 1988 [22].

The introduction of the recombinant form of human erythropoietin ushered in a new era in the management of anemia in end stage renal disease and later on in other non-dialysis expanded indications [18,23]. Epoetin alfa and beta are recombinant human erythropoietins. Darbepoetin alfa is a hyperglycosylated derivative of epoetin. The number of carbohydrate chains on the EPO molecule has an effect on serum clearance and determines the *in vivo* activity. The endogenous hormone and the epoetins (alfa and beta) all have three N-linked carbohydrate chains (Fig. 1), while darbepoetin alfa has five, which renders this compound longer-acting with a serum half-life of 25.3 versus 8.5 h of epoetin.

Epoetin is a man-made form of the human hormone erythropoietin [24]. It stimulates the bone marrow to produce red blood cells. It is used to treat anemia in patients with end-stage renal disease. It is also used to prevent or treat anemia caused by other conditions, such as acquired immunodeficiency syndrome (AIDS) or cancer. Epoetin is created by implanting a cloned erythropoietin-producing gene into chinese hamster ovary (CHO) cells. Brand names include Epogen® (Amgen), Procrit® (Ortho Biotech), Eprex® (Johnson & Johnson). Various stabilizers are used to keep the epoetin molecule stable. In the USA, epoetin alfa preparations (Epogen, Procrit) contain human serum albumin. However, in 1998 in Europe the stabilizer was switched to polysorbate 80 and glycine for epoetin alfa (Eprex). Epoetin beta (NeoRecormon, F. Hoffmann-La Roche) is a different formulation which uses a combination of stabilizers, including polysorbate 20, glycine, a complex of 5 other aminoacids, urea and calcium chloride. A rare but serious side effect of prolonged EPO-treatment is pure red cell aplasia (PRCA). It has been suggested that the increased incidence of red cell aplasia coincided with the removal of human serum albumin from the epoetin preparation. This caveat notwithstanding, epoetin alfa has been used for more than 15 years for the treatment of anemia and has proven to be safe and effective.

Darbepoetin alfa (Aranesp, marketed by Amgen) is a hyperglycosylated derivative of EPO with a longer serum

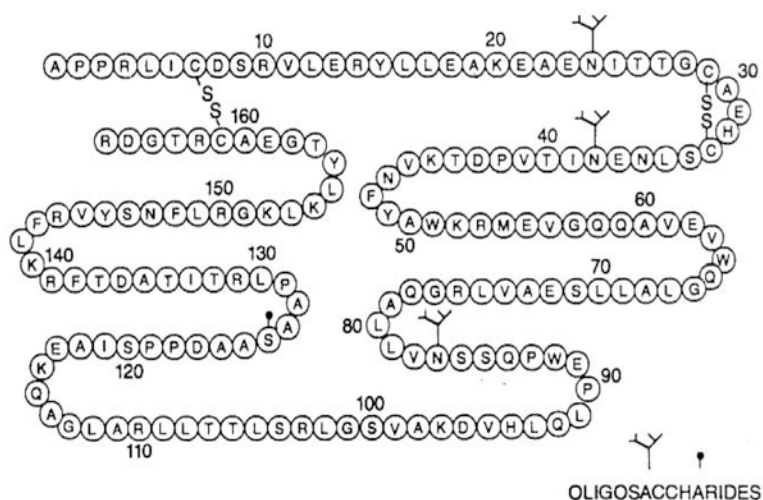


Fig. (1). Here is shown the molecular structure of human erythropoietin, which is a 30.4 kDa 165-amino-acid glycoprotein with 2 disulphide bonds, 3 N-linked carbohydrate (oligosaccharide) chains and ≤ 14 sialic acid residues.

half-life [19]. It was approved in September 2001 by the Food and Drug Administration for treatment of patients with chronic renal failure by intravenous or subcutaneous injection. It is produced in modified CHO cells. It differs from endogenous erythropoietin by containing two more N-linked oligosaccharide chains. It is an erythropoiesis stimulating 165-amino acid protein. Like EPO its use increases the risk of hypertension, thrombosis, or headache. Pre-existing hypertension contra-indicates the use of darbepoetin as does existing of a hematologic disease. Also like EPO it has the potential to be abused by athletes seeking ways to improve athletic performance.

Finally, a modified version of EPO, **carbamyated EPO** (CEPO) is a nonerythropoietic derivative of EPO which retains EPO's tissue protection but does not have the undesired effects of recombinant human EPO and is being tested in animal experiments [25].

The first clinical application of recombinant human EPO (rHuEpo) was in the treatment of anemia of chronic renal failure [9,26,27]. Therapy with rHuEpo is associated with an enhanced quality of life, cognitive function and activity level in end stage renal disease (ESRD) patients [26,27]. The indications for treatment with rHuEpo have been extended to patients with chronic renal failure not on dialysis, with a hematocrit less than 30%. Erythropoietin is now undergoing evaluation with promising results in a variety of new clinical settings, including anemia associated with congestive heart failure [5,8]. Other anemias with a variety of etiologies that can be corrected with use of EPO, comprise anemia in zidovudine-treated human immunodeficiency virus-infected patients, non-myeloid malignancies on chemotherapy, myelodysplastic syndromes, or anemia of prematurity [28-30]. Furthermore, EPO can be administered as prophylactic therapy to prevent anemia after surgery and to reduce blood transfusions in patients in intensive care units [31,32].

PHARMACOKINETICS

Erythropoietin (EPO) is a glycoprotein hormone with a molecular mass of 30.4 kDa, synthesized mainly in the kidney in response to hypoxia and stimulates the proliferation and differentiation of erythrocytic progenitors in the bone marrow [33]. The gene for EPO encodes a protein precursor of 193 amino acids, but the circulating EPO molecule has 165 amino acids (Fig. 1). Its isolation was achieved in 1977 and paved the way for cloning the gene and the industrial production of recombinant human EPO [20,21]. The plasma half-life of EPO ranges from 2 to 13 hours and its serum concentrations normally range from 6 to 32 U/L. Less than 10% of EPO is excreted in the urine.

Epoetin alfa is identical to endogenous human EPO, containing 165 amino acids, 3 N-linked carbohydrate chains and ≤ 14 sialic acid residues, $\sim 40\%$ carbohydrate and has a molecular weight of 30.4 kDa [33,34]. Its volume of distribution is similar to plasma volume. Its metabolism is believed to occur in the kidney, liver and bone marrow. It has a high affinity for binding with the EPO receptor with an inhibitory concentration of 138 pM. Its biological activity is equivalent to endogenous EPO. Its half-life is 8.5 h by intravenous administration and 16-19 h when given

transcutaneously. It has a 20-30% bioavailability and relative clearance after subcutaneous administration of 24.7 ml/h/kg. Time to reach peak serum concentration is estimated to be 16 ± 7.5 h when given subcutaneously in healthy volunteers and 18 h in dialysis patients.

Due to its increased (≤ 22) sialic acid-containing carbohydrate content, darbepoetin alfa has a higher molecular weight (37.1 kDa), a greater negative charge, and a \sim fourfold lower EPO receptor binding activity than rHuEpo. It also has a threefold longer circulating half-life (~ 25.3 h for intravenous and 33-48 h for subcutaneous administration) than rHuEpo in rats and dogs. In spite of its lower receptor binding, and perhaps counter-intuitively, darbepoetin alfa is significantly more potent *in vivo* than rHuEpo. Due to the pharmacokinetic differences, the relative potency of the two molecules varies as a function of the dosing frequency. Darbepoetin alfa is 3.6-fold more potent than rHuEpo in increasing the hematocrit of normal mice when each is administered thrice weekly, but when the administration frequency is reduced to once weekly, darbepoetin alfa is ~ 13 -fold to 14-fold more potent than rHuEpo. The pharmacokinetic and pharmacodynamic profiles and safety data for darbepoetin alfa demonstrate that it can be administered less frequently than epoetin (once-weekly instead of thrice weekly) in patients with chronic kidney disease receiving hemodialysis, thus simplifying anemia management. For darbepoetin, the time it takes the drug to reach maximum serum concentration following subcutaneous injection is 54 hours in dialysis patients and 86 hours in cancer patients.

The recommended dosing interval for epoetin alfa is thrice weekly, while for darbepoetin alfa is once weekly. Clinical experience and experimental data suggest that these two agents work in a similar manner with extended dosing intervals. Both agents need to reach the bone marrow to exert their effect on erythropoietin receptors. Although the half-life may not predict duration of action, the time to reach peak serum concentration will influence the onset of action of the agent.

ERYTHROPOIETIN AND HEART FAILURE

Anemia often complicates congestive heart failure (CHF) [2-7]. Since EPO has shown promising results in a variety of clinical settings complicated by anemia, its use has also been recommended in patients afflicted by CHF and anemia. Preliminary reports have been most encouraging and suggest that its use in this setting is beneficial and safe [5,8].

Anemia and Heart Failure

Despite remarkable advances in diagnosis and therapy over the past decade, the prognosis of patients with heart failure remains poor. A recent analysis from the Framingham Study demonstrated that the age-corrected one-year mortality for men has hardly changed in 50 years, having fallen from 30% in 1950 to only 28% in 1999, while in the 1990s the 5-year heart failure mortality just exceeded 50% [35]. It remains unknown whether this dismal outcome could be partly attributed to the underutilization of medications proven to be useful in CHF, especially beta-blockers and angiotensin converting enzyme (ACE) inhibitors, which unfortunately even today are reported to have limited

penetration in the community [36]. However, there may be several other important reasons for the persistence of such high mortality rates [35]. One such reason may be the high prevalence of untreated anemia among CHF patients.

Although anemia is a well-recognized important comorbidity in a variety of conditions, including coronary artery disease, its role in heart failure has only recently received attention. The attitude of physicians toward anemia while treating CHF patients has recently been studied [37,38]. Among patients with chronic stable heart failure seen at tertiary cardiology and internal medicine clinics in the US, anemia appeared in 29% of cases during the follow-up period. Once present, it persisted in 93.4% of cases and unfortunately it was included among the diagnoses in 11% of cases seen by internists and 4% of cases seen by cardiologists. Anemia etiology was sought in only 6% of all these anemic CHF patients and only 10% received therapy for it. The investigators concluded that anemia in CHF patients was under-recognized, under-diagnosed, and under-treated.

Recent reports examined anemia prevalence among patients with CHF. Anemia, defined as hemoglobin concentration ≤ 12 g/dL, was present in one third to one half of CHF patients in cohort and/or case-controlled studies conducted by Silverberg *et al.* [38]. Others provide more modest estimates that range from 4% to 30% [39-42]. In a large cohort of 12,065 patients with new-onset CHF, 17% had anemia according to Ezekowitz *et al.* [4]. The most common causes were anemia of chronic disease (58%) and iron deficiency (21%). Moreover, anemia was more common in older patients, women, patients with hypertension, or chronic renal insufficiency. The reasons for the differences in the prevalence of anemia observed in these various studies are probably the different definitions of anemia used, and the different populations studied. In summary, data collected from epidemiologic studies document that lower plasma hemoglobin levels in CHF are related to female gender, older age, poor kidney function, lower body weight, greater inflammation, and advanced disease status (based on left ventricular ejection fraction, exercise capacity, and mortality analyses) [43]. In general, one should expect that among CHF patients about one out of three has at least mild anemia.

Anemia is not only common among CHF patients but it increases with disease severity and unfavorably affects prognosis. Silverberg *et al.* found that among 142 heart failure patients treated at a specialized outpatient clinic, the worse the CHF the more prevalent and severe the anemia, such that 9.1% of cases of mild CHF, New York Heart Association (NYHA) class I, were anemic, with a mean hemoglobin level of 13.7 g/dl, while in contrast, in severe CHF, NYHA class IV, 79.1% were anemic with mean hemoglobin of 10.9 g/dl [8]. In a study by Horwich *et al.*, among patients with NYHA functional class III or IV, lower hemoglobin was related to an impaired hemodynamic profile and higher blood urea nitrogen and creatinine levels; patients in the lower hemoglobin quartiles were more likely to be at NYHA functional class IV and have lower peak oxygen consumption (VO_2 max); one-year survival was higher with increased hemoglobin quartile and low hemoglobin proved to be an independent predictor of mortality (relative risk 1.13, for each 1 g/dl decrease) [40]. Recent analysis of the

studies of left ventricular dysfunction (SOLVD) database also showed that anemia is an independent risk factor for mortality in patients with left ventricular dysfunction [39]. Similar results were reported by McClellan *et al.* [41], Anand *et al.* [42], Kosiborod *et al.* [44] and Ezekowitz *et al.* [4] in CHF patients, indicating that CHF severity is associated with significantly lower hemoglobin concentrations and that the presence of anemia is a strong independent predictor of increased morbidity and mortality.

The exact mechanisms underlying the pathogenesis of anemia in CHF are difficult to ascertain because pathophysiological correlates of CHF cannot only be a cause, but also a consequence of anemia (Fig. 2). The most probable cause of anemia in chronic CHF is renal insufficiency due to reduced cardiac output which can cause renal damage through prolonged renal ischemia [38]. In contrast to other populations with anemia of chronic disease, EPO levels are elevated in proportion to the severity of symptoms in patients with CHF [45,46]. Furthermore, a recent study suggested that elevated EPO levels in CHF patients are associated with an adverse prognosis independent of hemoglobin levels [47]. The authors also found a very modest inverse correlation between EPO and hemoglobin levels, indicating a blunted EPO response relative to the hemoglobin levels or even resistance to EPO in the bone marrow, possibly explaining the anemia observed in CHF patients. The increased production of EPO in CHF patients with anemia may reflect the presence of renal hypoxia and a compensatory attempt to augment O_2 delivery to peripheral tissues through erythrocytosis. Although endogenous EPO levels can be elevated above normal values in many anemic patients with CHF, this elevation is unlikely to compensate for the degree of prevailing renal hypoxia. Of course, if severe enough, coexisting renal failure will lead to EPO under-production. These consequences of chronic renal insufficiency that frequently complicates CHF can explain why recombinant human EPO administration has been already used in order to correct anemia in CHF patients.

The links between anemia, heart failure, and renal insufficiency have prompted Silverberg to coin the term *cardio-renal-anemia syndrome* [38]. This term implies that there are pathogenetic links among its 3 components (Fig. 2). In this syndrome anemia may cause chronic renal insufficiency or be caused by chronic renal insufficiency, anemia may cause CHF or be caused by CHF and CHF may cause renal insufficiency or be caused by renal insufficiency. The interaction among these three conditions takes the form of a vicious cycle that causes further deterioration of cardiac function, renal function and anemia. The fact that CHF could lead to prerenal azotemia is clear, and that heart and renal disease may share a common cause -- for instance, diabetes mellitus, hypertension or vascular disease -- is well-accepted. Chronic renal insufficiency also appears to be a risk factor in itself for atherosclerotic disease and for heart disease. This connection may depend on anemia. Analysis of the atherosclerosis risk in communities (ARIC study) showed that elevation of the serum creatinine above the normal range doubled the risk of coronary heart disease, but only when azotemia coexisted with anemia [48]. The stress of renal disease on a failing heart is undeniable, and anemia

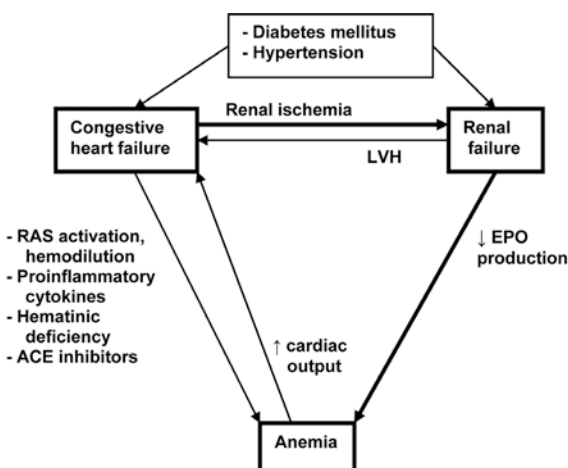


Fig. (2). Major pathogenetic links contributing to the cardio-renal-anemia syndrome (see text for discussion). LVH = left ventricular hypertrophy; RAS = renin-angiotensin-aldosterone system; ACE = angiotensin converting enzyme; EPO = erythropoietin.

can only aggravate the situation. Anemia is now confirmed as a risk factor for left ventricular hypertrophy in patients on chronic dialysis, and in patients with chronic renal insufficiency not yet on dialysis [49,50]. These observations extend to renal transplant recipients [51]. Left ventricular hypertrophy, in turn, is a risk factor for symptomatic heart disease, including heart failure and sudden death. The pathophysiologic connections between heart and kidney failure are thus close, well-studied, and definitely involve anemia.

The combination of CHF, chronic renal insufficiency, and anemia seems to have an additive effect on morbidity and mortality in CHF and renal insufficiency. This powerful interaction of these three factors is suggested by a recent study of a 5% sample of the Medicare population in the US. Each of these 3 factors increases the risk of death or end-stage renal disease by 50% to 100% and the 3 together raise the chances by up to 300% [38, 52]. This interaction between them is consistent with the presence of a vicious circle: *the cardio-renal-anemia syndrome*.

Renal dysfunction in heart failure is often complicated by diuretic resistance, anemia, hypotension, and the tendency to develop hyperkalemia. Diuretics relieve the symptoms of patients with cardio-renal failure, but these indispensable drugs may transform well-perfused dyspnea to low-volume prerenal azotemia. ACE inhibitors and aldosterone blockers that may slow the progression of heart failure may often also cause hyperkalemia. The patient with the cardio-renal-anemia syndrome will often be older and have a low ejection fraction, azotemia, and anemia. Dialysis may have been discussed as an option in patient care and the medication list is long and complicated. It is worthwhile to seek symptomatic relief in these patients without using dialysis. The recognition that anemia plays a major role in the cardio-renal syndrome not only elucidates its pathophysiology, but also offers the prospect of better treatment *via* correction of anemia.

Proinflammatory Cytokines and EPO Resistance

Since many anemic CHF patients have a normal serum creatinine level, it is unlikely that renal dysfunction entirely explains anemia seen together with CHF. Usually renal anemia develops in chronic renal dysfunction with serum creatinine over 3.5 mg/dl or a creatinine clearance below 30 ml/min. But mere azotemia will not explain the 14% of subjects in the study by McClellan *et al.* who had a hematocrit of less than 30%, well below what is expected for the average serum creatinine of 2 mg/dl in that group [41]. The inflammatory milieu of heart failure seems to contribute to the genesis of anemia in these patients.

Depressed hemoglobin levels are frequently seen in inflammatory conditions; substantial evidence implicates tumor necrosis factor (TNF) and interleukins (IL) 1 and 6 in the disruption of erythropoiesis in several ways [53]. Proinflammatory cytokines seem to be pivotal triggers of anemia in CHF. The damaged heart secretes TNF-alpha, which can cause anemia in three ways: a) by reducing EPO production in the kidneys, b) by interfering with EPO activity at the level of the bone marrow (EPO resistance), and c) by inhibiting the release of iron from the reticulo-endothelial system so that it cannot get to the bone marrow to be utilized in hemoglobin production [54-56]. In CHF patients, an inverse relationship has been demonstrated between cytokines (TNF and soluble TNF receptors) and plasma hemoglobin levels [57]. In a recent important study, Iversen *et al.* demonstrated that the induction of CHF in mice attenuated the bone marrow pro-erythroblast population (<40% of control) and proliferative capacity (<50% of control). A 3-fold increase in pro-erythroblast destruction was observed, and significantly correlated with increased TNF mediated apoptosis [58]. Supportive evidence exists though for proinflammatory cytokines acting as a causative link between CHF and anemia.

Hematinic Deficiency

A number of abnormalities in CHF predispose to iron and other hematinic deficiencies. There may be reduced intestinal iron uptake associated with poor nutrition, cardiac cachexia, uremic gastritis and malabsorption. Congestive heart failure patients often have proteinuria, and both EPO, iron, and transferrin can be lost in significant amounts in the urine also contributing to the anemia [59]. Treatment of the disease underlying CHF, principally coronary artery disease, with agents such as aspirin and warfarin can contribute to iron loss and anemia due to gastrointestinal blood loss. Ezekowitz *et al.* reported a diagnosis of iron deficiency in 21% of anemic CHF patients [4]. Malnutrition in CHF also threatens folate and vitamin B₁₂ levels.

Hemodilution

A reduced hemoglobin concentration in CHF may also be a result of hemodilution. Congestive heart failure is characterized by overall tissue ischemia and activation of the sympathetic system, the renin-angiotensin-aldosterone system and vasopressin. Such activation leads to sodium and water retention [60,61]. Two recent studies involving CHF patients have reported hemodilution to be present in 46% [62] and 40% [63] respectively. However in the majority of cases there is also a reduced red blood cell volume [62].

ACE Inhibitors

Treatment of heart failure itself could cause anemia, and ACE inhibitors, being standard therapy, may cause the hematocrit to fall by 2% to 5%. High doses of ACE-inhibitors have been demonstrated to impair the response to erythropoietin treatment in hemodialysis patients [64,65]. Treatment with ACE inhibitors may also inhibit the synthesis of endogenous EPO [66]. The role of ACE inhibitors needs further investigation in this regard.

Erythropoietin Therapy in Congestive Heart Failure

Studies with rHuEpo

Drugs used in the treatment of CHF include digoxin, diuretics, ACE inhibitors, angiotensin-receptor blockers, aldosterone antagonists, and beta-blockers. The latter four categories of therapeutic agents have been associated with improved survival in patients with heart failure according to a multitude of studies over the past decade. However, these medications are not without side effects, including the risk of hyperkalemia that can occur in patients treated with spirono-

lactone [67]. Despite aggressive therapy with all the conventional medications at the accepted doses, heart failure continues to carry a poor long-term prognosis, also limiting quality of life. Better treatment modalities are really needed. As anemia is associated with increased morbidity and mortality in CHF, correction of anemia emerges as a promising novel method to improve patient outcomes.

There is a wealth of clinical data demonstrating the positive benefits of anemia correction in patients with renal disease, including improvement in cardiac function [9,68-70]. In chronic diseases such as end stage renal failure, malignancy, or HIV infection, treatment with rHuEpo increases hemoglobin concentration, decreases the need for blood transfusion and improves outcomes [71]. The pathophysiology of anemia in CHF also suggests EPO as a logical therapeutic step.

There have been preliminary reports by at least two groups that have used rHuEpo to correct anemia in patients with advanced CHF (Table 1). Silverberg *et al.* performed a retrospective evaluation of the significance of anemia in patients with chronic CHF and prospectively determined the

Table 1. Published Studies for the Assessment of Erythropoietin Administration to Patients with CHF and Anemia

Study	Number of patients	NYHA class	Regimen	Hb increase	Clinical outcome
Silverberg <i>et al.</i> (2000) [8]	26	IV	-rHuEpo * 2000 IU/ week (target Hb: 12 g/dl) -IV Fe 200 mg/week (target ferritin 400 µg/L or FeS>40% or Hb>12 g/dl)	2 g/dl	-NYHA class improvement -LVEF ↑ -Dramatic ↓ of hospitalizations
Silverberg <i>et al.</i> (2001) [72]	32	III, IV	-rHuEpo * 4000 IU/week (target Hb: 12.5 g/dl) -IV Fe 200 mg / 2 weeks (target ferritin 400 µg/L or FeS>40% or Hb>12.5 g/dl)	2.6 g/dl	-NYHA class improvement -LVEF ↑ -Hospitalization days ↓ -Diuretic dose ↓ -Stabilization of renal function
Silverberg <i>et al.</i> (2003) [73]	84 diabetics & 95 non-diabetics	III, IV	-rHuEpo * 4000-5000 IU/ 1-3 weeks (target Hb: 12.5 g/dl) -IV Fe 200 mg / 1-2 weeks (target ferritin 500 µg/L or FeS>40% or Hb>12.5 g/dl)	2.7 g/dl	Similarly in diabetics and non-diabetics: -NYHA class improvement -Reduced breathlessness and/or fatigue -LVEF increase -Dramatic ↓ of hospitalizations -Stabilization of renal function
Mancini <i>et al.</i> (2003) [63]	23	III, IV	-rHuEpo * 15000-30000 IU / week, ferrous gluconate 325 mg/day, folate 1 g/day	3.3 g/dl	-Improved functional capacity indices (V _{o2} max, 6 min walk test, exercise time) -Improved quality of life
Silverberg <i>et al.</i> (2005) [74]	78		-rHuEpo ** 5000-10000 IU / week, -IV Fe 200 mg / week (target ferritin 700 µg/L or FeS >40% or Hb>13 g/dl)	3.3 g/dl	-NYHA class improvement -LVEF increase -↓ of hospitalizations -Stabilization of renal function

CHF= congestive heart failure; Fe= iron; FeS= % Fe saturation; Hb= hemoglobin; LVEF= left ventricular ejection fraction; NYHA= New York Heart Association; rHuEPO= recombinant human erythropoietin

* epoetin alpha

** epoetin beta

effect of its correction on cardiac and renal function [8]. Among 142 patients suffering from CHF, the mean hemoglobin levels were 13.73 and 10.9 g/dl for NYHA class I and IV respectively. Among 79 patients who had anemia (hemoglobin <12 g/dl), 53 were at NYHA class IV CHF. Fifty-eight patients had chronic renal insufficiency (serum creatinine >1.5 mg/dl), with mean serum creatinine 1.18 mg/dl in class I and 2.0 mg/dL in class IV patients. Twenty-six patients who had chronic NYHA class IV CHF, refractory to maximally tolerated medical therapy and hemoglobin <12 g/dL, took part in the interventional arm of the study. Treatment consisted of subcutaneous rHuEpo and intravenous iron and lasted for a mean of 7.2 ± 5.5 months. Recombinant human EPO was given once a week at a starting dose of 2,000 IU per week subcutaneously, and the dose was increased or decreased as necessary to achieve and maintain a target hemoglobin of 12 g%. Intravenous iron supplement (a ferric sucrose product) was given in a dose of 200 mg in 150 ml saline over 60 min every week until the serum ferritin reached 400 $\mu\text{g/L}$ or the percent iron saturation reached 40% or until the hemoglobin reached 12 g/dl. Intravenous iron was then given at longer intervals as needed to maintain these levels. Mean hemoglobin, serum ferritin, and iron levels increased significantly with treatment. The mean dose of rHuEpo was 5227 ± 455 IU/week, and the mean dose of iron was 185.1 ± 57.1 mg/month. After treatment, the daily dose of oral furosemide decreased significantly (from 200.9 ± 120.4 mg/day before to 78.3 ± 41.3 mg/day after the intervention) and so did the monthly requirement of intravenous furosemide (164.7 ± 178.9 mg/month before to 19.8 ± 47.0 mg/month after). The NYHA class decreased (from a mean of 3.66 ± 0.47 to 2.66 ± 0.70), and the mean left-ventricular ejection fraction (LVEF) increased significantly from baseline (from $27.7 \pm 4.8\%$ to $35.4 \pm 7.6\%$). The number of hospitalizations also decreased dramatically (by 91.7%). Baseline serum creatinine was 2.59 ± 0.77 mg/dl, and changes over the treatment period were not significant. No adverse effects were reported. The results of this trial demonstrated that rHuEpo can improve cardiac function and functional status, stabilize renal function and decrease the need for diuretics and hospitalization.

The same group of investigators subsequently performed an open-label, randomized, controlled study to assess the effects of rHuEpo on cardiac and renal function and days of hospitalization in patients with chronic CHF [72]. It comprised 32 CHF patients of NYHA functional class III or IV, already treated with maximally tolerated doses of medications for heart failure for at least 6 months. They all had LVEF $\leq 40\%$ and hemoglobin levels between 10 and 11.5 g/dl. Patients were randomized to receive either subcutaneous rHuEpo and intravenous iron or no treatment for anemia. There was no significant difference in mean serum creatinine levels between the 2 groups at baseline (control group 1.4 ± 0.9 mg/dl, treatment group 1.7 ± 0.8 mg/dl). Over a mean of 8.2 ± 2.6 months, no patient in the active treatment group died of CHF-related illnesses, while four patients died in the no treatment group. The NYHA class in the treatment group decreased, but increased significantly in the control group. Left ventricular ejection fraction decreased significantly in the control group, but increased significantly in the treatment group. The dose of

oral furosemide used in the treatment group decreased significantly. Conversely, the dose of oral furosemide required by the control group increased. Compared to a similar period prior to this intervention, the number of hospitalization days increased significantly in the control group and decreased significantly in the treatment group.

A third study by the same group examined the correction of anemia in diabetic and non-diabetic patients with chronic CHF refractory to medical treatment and chronic renal failure [73]. Anemia was treated in the same way as in the earlier study [72], with the exception that rHuEpo was initiated at a dose of 4000–5000 units weekly and the ferritin target was 500 instead of 400 $\mu\text{g/L}$. Eighty-four patients with type 2 diabetes mellitus and 95 non-diabetic patients were included and followed prospectively for a mean duration of 11.8 ± 8.2 months. Left ventricular ejection fraction increased significantly, while NYHA class and number of hospitalizations decreased significantly in both groups. The degree of fatigue and shortness of breath (as assessed by a visual analog scale, range from 0 = normal breathing/strength to 10 = extreme fatigue/shortness of breath) improved significantly in both groups. Ten patients without diabetes and 8 diabetic patients died during the intervention period. This study demonstrated once again that rHuEpo had a favorable effect in CHF, and moreover that such an effect was independent of the presence of diabetes mellitus.

Another randomized, controlled trial enrolled 23 anemic patients with chronic CHF, at NYHA class III or IV, receiving stable medication regimens for 4 weeks [63]. They all had hematocrit <35%, serum creatinine <2.5 mg/dl and erythropoietin level <100 mU/ml. Among parameters measured at baseline and the end of therapy were blood parameters (hemoglobin, hematocrit, plasma volume) and exercise parameters (peak oxygen consumption [$\text{V O}_2 \text{ max}$], exercise duration, 6-minute walk). Patients were randomized in a single-blind fashion to receive either co-therapy with rHuEpo (15000–30000 IU per week), ferrous gluconate (325 mg/day) and folate (1 mg/day) or placebo. The average duration of treatment in the rHuEpo group was 70 ± 11 days. EPO levels before treatment were elevated (normal 4.1–19.5 mU/mL), but did not differ between groups (control 32 ± 16 mU/ml, treatment 24 ± 14 mU/mL). Serum creatinine was also similar (mean 1.6 mg/dl in both groups). Administration of rHuEpo was well tolerated. There were significant increases in hemoglobin (from 11.0 ± 0.5 to 14.3 ± 1.0 g/dl), $\text{VO}_2 \text{ max}$ (from 11.0 ± 1.8 to 12.7 ± 2.8 ml \times min⁻¹ \times kg⁻¹) and exercise duration (590 ± 107 to 657 ± 119 sec) in the rHuEpo group. No significant changes were noted in the control group. In the active treatment group, 12 of 15 patients experienced improved quality of life versus only 1 of 8 patients in the control group. The anemia in about 40% of these patients was dilutional and secondary to increased plasma volume. In the dilutional anemia sub-group, red blood cell volume seemed to replace the plasma volume excess after rHuEpo therapy, thus providing an explanation for the similar results concerning improved submaximal and maximal exercise capacity in this patient population in the dilutional and non-dilutional anemia subgroups [63].

Finally, Silverberg *et al.* in another most recent study [74] treated 78 patients with symptomatic CHF and anemia

(hemoglobin <12.0 g/dl) with subcutaneous epoetin beta and, if necessary, intravenous iron sucrose. EPO therapy resulted in anemia correction coupled with significant improvement in NYHA class and cardiac function together with a reduction in hospitalization rate, while renal function was maintained stable in most patients.

Overall, the number of reports about the use of erythropoietin for the treatment of anemia in heart failure patients is still limited (Table 1). Considering the outcomes of the first treatment efforts with rHuEpo, more questions are raised than those answered. From studies published so far it has been demonstrated that the use of rHuEpo in chronic CHF class III and IV patients with anemia (defined as hemoglobin <12 g/dL), refractory to maximally tolerated medical management, improves symptoms and decreases the number of hospitalizations. These studies had all small sample sizes, and differed according to the duration of follow up, their design, the severity of anemia and CHF in the populations studied and their end-points. Thus, it is difficult to compare their results. In all of them EPO was combined either with intravenous iron or with oral iron and folate. Some of the improvements observed could be attributed to these other treatments but the magnitude of their impact is elusive. The treatment target of hemoglobin was set at 12–12.5 g/dL, based on epidemiology studies.

The optimal goal for hemoglobin correction needs further research efforts in order to be defined. It is important to mention in this regard the study of Besarab *et al.* [75]. This large randomized controlled trial of rHuEpo in 1,233 dialysis patients, many with symptoms of heart failure, failed to show a benefit of aiming for higher rather than lower hematocrit values. On the contrary, the higher target hematocrit group had increased cardiovascular mortality and morbidity. Rapid and near normal correction of the hematocrit of patients to a mean of 42% increased cardiovascular events compared with those maintained at a hematocrit of 30%, despite the fact that within each randomized group a higher hematocrit was associated with a lower cardiovascular event rate. By increasing the hemoglobin concentration, maximum oxygen tissue delivery increases, but at the cost of increased blood viscosity and a potential for thrombotic complications. Although the interpretation of the above findings is uncertain, there is increasing evidence that correction of anemia to a hematocrit of 36% is safe [76,77], or a more flexible approach with individualized treatment targets may be preferable [78].

Predicting which patient with anemia and CHF will respond to erythropoietin therapy is also important. Indicators that might foretell a response to erythropoietin in non-renal applications have been studied. The combination of low baseline endogenous serum erythropoietin concentrations (<50-100 mU/ml), a low ratio of observed-to-predicted serum erythropoietin values, a sufficient increase in hemoglobin after two weeks of erythropoietin treatment (>0.3 to 0.5 g/dl), and a serum ferritin concentration of <400 ng/ml has been evaluated in various algorithms [79,80]. Additional indicators that could predict a positive response to erythropoietin may be needed in anemic patients suffering from CHF. The small number of studies conducted so far demonstrated benefits concerning the functional capacity of

anemic CHF patients of advanced NYHA class. The mechanism by which EPO improves exercise capacity is not known. In sports medicine, the mechanism has been presumed to be from increased hemoglobin concentration leading to increased oxygen delivery. In disease states where oxidative stress is increased, however, hemoglobin may also reduce oxidative stress by scavenging for O₂ free radicals. This may improve endothelial function and increase the rate of oxygen delivery. In end stage renal patients, EPO has been shown to improve skeletal muscle function and O₂ use as well as endothelial function [75,81]. It has been suggested by *in vitro* evidence that even EPO itself may reduce oxidative stress by direct cellular effects independent of its effect on hemoglobin concentration [82].

Concerning functional capacity improvements with EPO administration, it should be considered whether they are to be attributed to its ability to increase hemoglobin or to other recently discovered biologic effects of this glycoprotein conferring cardioprotection (Fig. 3). EPO is a hypoxia-induced cytokine, with its receptors spread in the cardiovascular system, including endothelial cells, smooth muscle cells and cardiomyocytes. It has been demonstrated that erythropoietin may exert anti-apoptotic, mitogenic, and angiogenic effects. These protective actions of EPO on cardiomyocytes are documented *in vivo* and *in vitro* with the use of cardiac ischemia – reperfusion models [12]. Recently, carbamylated EPO (CEPO), a derivative of erythropoietin devoid of erythropoietic activity, has been also shown to protect the myocardium from ischemia - reperfusion injury, further supporting the fact that EPO has significant salutary effects beyond erythropoiesis [25]. Such effects on cardiomyocytes may be part of the explanation for the reported functional improvement of CHF patients with anemia.

Since such improvement was observed in patients with severe and long-standing CHF another issue raised is whether EPO administration would be clinically beneficial at the early stages of the heart failure syndrome, for example even as early as just after a myocardial infarction. Other questions should also be answered: can EPO benefit CHF patients of any underlying etiology? Would it improve patients with CHF but without anemia? Is rHuEpo or its derivatives more appropriate for such therapies? However, since the first few studies of EPO treatment for heart failure report morbidity reduction for patients with advanced CHF and anemia after rHuEpo administration, the first in a series of questions that deserves an answer is whether EPO can provide survival benefit as well.

Larger and adequately powered studies are needed. Three prospective randomized trials are expected to further elucidate the magnitude of cardiovascular benefits that EPO could provide. The Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE) Trial will compare the effect of early (when hemoglobin falls within the range of 11 -12.5 g/dl, to a target of 13-15 g/dl) versus late (when hemoglobin has dropped to < 10.5 g/dl, to a target of 10.5-11.5 g/dl) anemia correction, among chronic kidney disease patients, upon left ventricular mass index (LVMI) and other cardiovascular end points [83]. The Trial to Reduce Cardiovascular Events with Aranesp Therapy

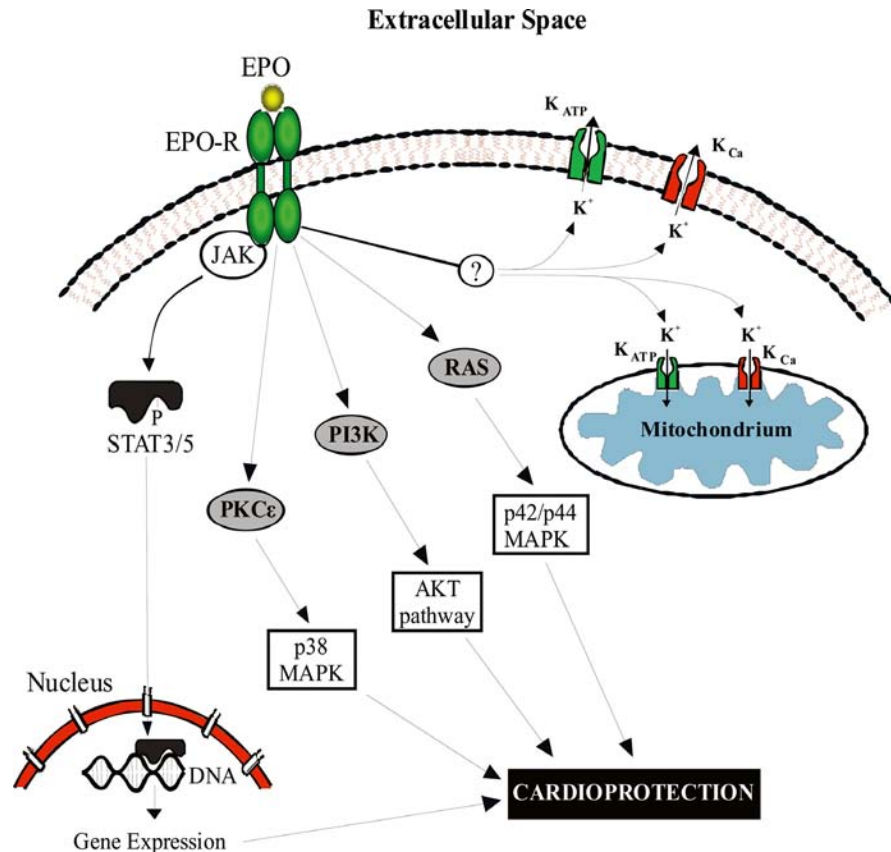


Fig. (3). Schematic representation of signalling pathways activated by erythropoietin (EPO) binding to erythropoietin receptor (EPO-R) which mediate the conferred cardioprotection.

JAK: Janus-tyrosine kinase; STAT: signal transducer and activator of transcription; PKC ϵ : ϵ isoform of protein kinase C; MAPK: mitogen-activated protein kinase; PI3K: phosphatidylinositol-3-kinase; AKT: protein kinase B; K_{Ca}: calcium activated potassium channels; K_{ATP}: ATP-sensitive potassium channels.

(TREAT) will assess the effect of darbepoetin alpha (Aranesp) administration in patients with chronic kidney disease and type II diabetes mellitus on cardiovascular mortality and other cardiovascular end points [83]. In the Studies of Anemia in Heart Failure Trial (STAMINA HeFT), 250 patients with chronic CHF and hemoglobin <12 g/dl will be randomized to receive darbepoetin every 2 weeks for one year; exercise treadmill tests will be used to assess changes of functional status as a surrogate end point, since the study is not powered enough for mortality per se [84]. In conclusion, erythropoietin is a new promising agent in the therapeutic armamentarium of cardiovascular medicine. Ongoing active research efforts are expected to define its exact future role, with priority given to the management of chronic congestive heart failure (Fig. 4).

PLEIOTROPIC EFFECTS OF ERYTHROPOIETIN

In addition to classic vascular endothelial growth factor (VEGF), another proangiogenic growth factor currently receiving attention in cardiovascular research is the hematopoietic cytokine erythropoietin (EPO). EPO has pleiotropic effects well beyond the maintenance of red blood cell mass [10-17] (Fig. 5). In the embryo, EPO is a major regulator of vascular formation and organ growth; EPO receptors are found in almost every embryonic tissue. EPO receptors also

exist in many adult tissues, and the notion of autocrine or paracrine EPO systems has been raised. EPO also acts on endothelial cells. Human endothelial cells respond to EPO by differentiating into vascular structures. In addition, EPO has important cytoprotective effects, including protection from ischemic injury and inhibition of apoptotic death-related pathways. Low-dose treatment with the long-acting EPO analogue, darbepoetin, confers vascular and tissue protection that is associated with persistent stimulation of the pro-survival Akt signaling pathway. The use of recombinant human erythropoietin or analogues may have utility in preventing ischemia-related progressive vascular injury and organ failure.

Recent studies have identified multiple paracrine/autocrine functions of EPO coordinating local responses to injury by maintaining vascular autoregulation and attenuating primary apoptotic and secondary inflammatory causes of cell death. Experimental data support a role for EPO in repair and regeneration of brain and spinal cord tissue following injury [85]. Similarly, there is evidence that EPO prevents apoptosis of cardiomyocytes and attenuates post-infarct deterioration in hemodynamic function, suggesting that EPO is possibly a tissue-protective cytokine. EPO can stimulate proliferation of myoblasts to expand the progenitor population during

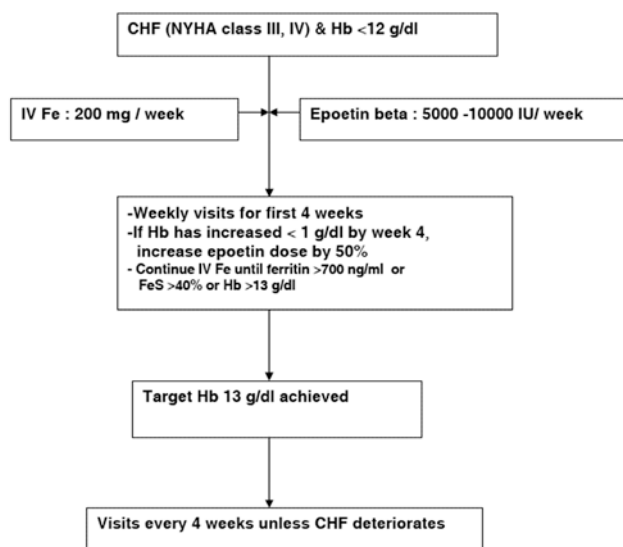


Fig. (4). Suggested treatment for anemia in CHF patients [74].

CHF= congestive heart failure; Fe= iron, FeS = % Fe saturation; Hb= hemoglobin; IV= intravenous; NYHA= New York Heart Association.

differentiation and may have a potential role in muscle development or repair [14].

A. Neuroprotection

Erythropoietin is expressed by several cell types in the nervous system including neurons, glial cells and endothelial cells while binding sites in the human brain have been localized in temporal cortex, hippocampus, cerebellum and amygdalae [86,87]. EPO has been shown to exert a neurotrophic effect on cholinergic neurons influencing their differentiation, maintenance and regeneration [88], while EPO and its receptor are expressed in the developing human central nervous system and decrease apoptotic neuronal cell death under hypoxic conditions [87].

EPO confers neuroprotection following ischemic, hypoxic, metabolic, neurotoxic and excitotoxic stress in the nervous system. The neuroprotective role of EPO following cerebral ischemia has been demonstrated in several experimental models [89-94]. It protects neurons from hypoxia and glutamic acid-induced toxicity [92,95-96], while its direct infusion into the brain reduces neurologic dysfunction in experimental models of stroke [89,92,97-98]. Furthermore, infusion of soluble EPO receptor which binds to the endogenous EPO, antagonizing its cellular effect, resulted in enhanced neuronal damage and learning ability, thus confirming the crucial role of EPO in neuronal survival following ischemic insult [97]. It is interesting that the abovementioned cytoprotective properties of EPO have triggered extensive research in several disease entities where

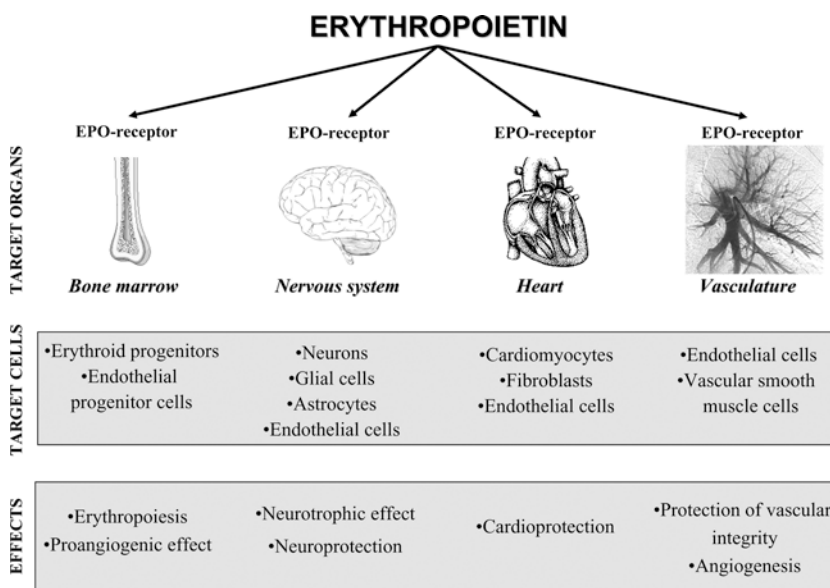


Fig. (5). Schematic illustration of the effects of erythropoietin on target cells and organs.

EPO is believed to be a suitable candidate for therapeutic exploitation. Bianchi *et al.* have shown in a recent trial that intraperitoneal EPO administration prevents and even reverses to some extent diabetic neuropathy in rats with streptozocin-induced diabetes [99]. Furthermore, EPO may increase the proliferation of neural progenitor cells and might play a therapeutic role in neurodegenerative disorders.

The underlying mechanism of EPO-mediated neuroprotection, though not fully clarified, is largely attributed to its anti-apoptotic effect. Erythropoietin binding with its receptor (EPO-R) results in homodimerization, conformational changes and activation of EPO-R [100], as well as autophosphorylation of a key-molecule Janus-tyrosine kinase-2 (JAK-2) [101]. JAK-2 phosphorylation mediates the phosphorylation of several downstream molecules such as the signal transducer and activator of transcription protein (STAT-5), phosphatidylinositol-3-kinase [PI(3)K], mitogen activated protein kinase (MAPK), inhibitor of the transcription factor NF κ B (I κ B) [102] which in turn activate diverse intracellular signal transduction cascades with the resultant expression of protective anti-apoptotic end-effectors. Apart from its anti-apoptotic role in neuroprotection [98,102,103], EPO diminishes the production of neurotoxic substances such as glutamate and reactive oxygen species [101,102], modulates neurotransmission, reverses vasospasm [104,105], preserves endothelial cell integrity [10,106-107], stimulates angiogenesis [108], modulates inflammation [90, 109,110] and enhances the recruitment of stem cells [111].

Evidence derived from *in vivo* and *in vitro* experimental models [104] supporting the neuroprotective role of EPO has raised the issue of its therapeutic exploitation in order to prevent neuronal loss in a wide range of central nervous system (CNS) disorders such as ischemic stroke, Parkinson's disease, Alzheimer disease, amyotrophic lateral sclerosis, multiple sclerosis, and neurotrauma. A recent, double-blind, randomized, proof-of-concept trial demonstrated the safety and efficacy of EPO in the treatment of patients with ischemic stroke. Intravenous high dose recombinant human EPO (rHuEpo) administration in acute ischemic stroke patients within 8 hours of the onset of symptoms was well tolerated and was associated with a significantly better recovery at 1-month follow-up as indicated by follow-up and outcome scales, sequential lesion size assessment by magnetic resonance imaging (MRI) and measurement of the serum marker of brain injury S100 [112]. It should also be emphasized that intravenous administration of rHuEpo did not result in elevated hematocrits and also led to a 60 to 100 fold increase in cerebrospinal fluid EPO levels which demonstrated that this 30.4-kDa glycoprotein can cross the blood-brain barrier when administered in high doses. The results of this trial suggest that EPO might represent a new therapeutic tool in patients with stroke, differing from the current dogma of reopening the feeding artery which is not feasible in the majority of strokes. Its administration protects potentially viable cells by inhibiting death signals and activating survival signal transduction pathways. Under this scope EPO is not a competitor but rather a complement to recombinant tissue plasminogen activator (r-tPA) since it could be used in combination with r-tPA in suitable candidates aiming at protecting viable cells and reducing

reperfusion injury, and furthermore as an alternative in patients excluded from the use of thrombolytic treatment.

The major limitation of EPO administration targeting neuroprotection is its hematopoietic properties which result in increased hematocrit levels and predispose patients to stroke and thrombosis. Thus the dissociation of the cytoprotection and hematopoiesis induced by EPO represents a major challenge. Asialoforms of EPO have a shortened half-life and have been demonstrated to remain neuroprotective without changing hemoglobin levels [91]. Leist *et al.* generated mutants of EPO, such as the carbamylated EPO (CEPO), that preserve the original neuroprotective properties of EPO without triggering hematopoiesis upon chronic dosing in different animal species. These compounds conferred protection comparable to that of EPO against stroke, spinal cord injury, diabetic neuropathy, and experimental autoimmune encephalomyelitis [113].

In conclusion, experimental data and a proof-of-concept clinical study demonstrate that erythropoietin and the EPO mutants which are devoid of erythropoietic side effects might open novel therapeutic avenues in clinical practice against several CNS diseases including ischemic stroke.

B. Cardioprotection

A sizeable amount of evidence demonstrating beneficial effects of EPO in experimental models of CNS disorders combined with the presence of EPO receptors in several cell types in the heart (endothelial cells [114,115], fibroblasts [116], cardiomyocytes [116-118]) has provided the rationale for investigating potential cardioprotective properties of this cytokine in the setting of myocardial ischemia (Fig. 3).

EPO has been shown to increase cell survival following hypoxia, oxidative stress or simulated ischemia in cultures of H9C2 myoblasts [13], adult rat cardiomyocytes [17] and neonatal rat ventricular myocytes [119]. The protection of cardiomyocytes *in vitro* validates that EPO possesses direct cytoprotective properties which are not related to changes in hematocrit levels (pleiotropic effects). Furthermore, several *in-vivo* studies have shown that pretreatment with EPO decreases infarct size and improves post-ischemic functional recovery [13,17,116,118-123]. Single systemic administration of EPO immediately after coronary artery ligation in rats resulted in decreased infarct size after 8 weeks at least partly due to an early direct anti-apoptotic effect of EPO and attenuated left ventricular functional impairment and adverse remodeling as assessed by repeated echocardiography [122]. Beneficial effects have been observed following chronic pretreatment with EPO [17,116,121], as well as when EPO is administered immediately before [17,120] or even at the time of the ischemic insult [13,17,25,116,119,123]. However, therapeutic interventions that should be applied concurrently with the advent of an ischemic episode have practical limitations. Parsa *et al.* have elegantly demonstrated that EPO single-dose administration during reperfusion significantly reduced infarct size and attenuated apoptosis, albeit to a lesser extent in comparison to pre-treatment with EPO [116]. The issue of the optimal timing of EPO administration in order to achieve maximal protection has also been addressed by Lipsik *et al.* [123], who demonstrated that EPO

administration after the onset of reperfusion reduced infarct size 24 hours following coronary occlusion in rats. This working group also reported that apoptosis was significantly attenuated in groups treated with EPO at the start of ischemia and after the onset of reperfusion and to a lesser extent in the group pre-treated with EPO [123]. Fiordaliso *et al.* demonstrated in a recent study that the novel carbamylated EPO analog (CEPO) which is nonerythropoietic and devoid of the undesirable effects of EPO, also exhibits cardioprotective properties. CEPO administration in rats on a daily basis for 1 week, with the first dose administered 5 min before reperfusion, decreased cardiomyocyte loss, prevented compensatory hypertrophy, reduced left ventricular wall stress and improved left ventricular hemodynamic parameters following myocardial infarction. The conferred cardioprotection was at least partly due to attenuation of cardiomyocyte apoptosis [25].

Mechanisms of Cardioprotection

Antiapoptotic Effect

The cardioprotection conferred by EPO is mediated by activation of well-known survival pathways that attenuate apoptosis (Fig. 3). Several studies have validated the early anti-apoptotic effect of EPO by elegantly demonstrating the activation of survival anti-apoptotic pathways as well as the reduction of TUNEL-positive nuclei following EPO administration in experimental models of ischemia and reperfusion [17,122].

Cai *et al.* showed in an in-vitro rat heart model that low-dose rHuEpo infusion conferred immediate cardioprotection against ischemia-reperfusion injury which was dependent on the activation of the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signal-transduction pathway. Recombinant human EPO enhanced the phosphorylation of p70 S6 kinase (p70^{S6K}), a downstream molecule of AKT and blocked apoptotic DNA laddering and caspase 3 activation in a phosphatidylinositol-3-kinase-dependent manner [120]. These experimental data demonstrated that EPO administration immediately before the ischemic insult is associated with cardioprotection through activation of the well-established PI3K/AKT survival pathway which inhibits apoptosis [120,124]. Parsa *et al.* have demonstrated in an *in vivo* rabbit heart model that EPO administration for 12 hours activates the Jak/STAT, ERK/MAPK, and PI3K/Akt kinase pathways [13]. The immediate activation of multiple cell survival signal transduction pathways, such as JAK/STAT, Ras-p42/44MAPK and PI3K-AKT, in response to EPO treatment has also been validated in an infant rabbit heart model of ischemia and reperfusion [125]. These pathways are also activated as an endogenous means of cardioprotection following ischemia-reperfusion, validating that EPO-mediated pharmacological preconditioning and ischemic preconditioning seem to share common underlying mechanisms [126].

Shi *et al.* delineated another survival signal-transduction pathway that is activated by EPO and results in enhanced functional recovery following ischemia-reperfusion in infant rabbit hearts. The mechanism underlying EPO-induced cardioprotection was reported to involve activation of PKC ϵ ,

p38 MAP kinase and p42/44 MAP kinase as well as activation of calcium activated (K_{CA}) and ATP-sensitive potassium channels (K_{ATP}), thus promoting potassium efflux from cytosol into the mitochondria and outside the cell [127].

Protection of Vascular Integrity

The attenuation of post-ischemic injury is directly related to the protection of endothelial cells and the maintenance of endothelial function, vascular integrity and flow in the coronary vasculature. EPO enhances the survival of endothelial cells against ischemic vascular injury by preventing apoptosis. In endothelial cells (EC), EPO induced cytoprotection is partly dependent on AKT1 activation. AKT1 modulates mitochondrial membrane potential thus preventing mitochondrial membrane depolarization and the subsequent cytoplasmic release of cytochrome c. The prevention of cytochrome c release in the cytoplasm explains the ability of EPO to prevent the activation of caspases 8, 1 and 3 which mediate apoptosis by promoting DNA fragmentation and membrane PS exposure [10,128].

Proangiogenic Effect

A fundamental response to tissue ischemia is the activation of endogenous mechanisms aiming at the formation of neovasculature that will supply oxygen and nutrients to the ischemic tissue. Postnatal neovascularization requires recruitment of endothelial progenitor cells which may differentiate into endothelial cells in-situ [129], proliferation of pre-existing endothelial cells, their migration to the ischemic region and ultimately the formation of functional vasculature. EPO activates several key-steps of the above-mentioned angiogenic process. Endothelial cells express EPO receptors [114] and EPO has been demonstrated to stimulate their proliferation, migration and differentiation into vascular structures [115,130]. In endothelial cell cultures derived from human adult myocardial tissue, rHuEpo significantly accelerated capillary outgrowth exhibiting equal angiogenic potential as that of vascular endothelial growth factor [131]. Furthermore, the Akt/PKB signal transduction pathway, which is activated by EPO, plays a critical role in the regulation of angiogenesis and neovascularization in ischemic tissue [132,133].

Apart from affecting mature endothelial cells, EPO stimulates the mobilization of endothelial progenitor cells from the bone marrow as well as their proliferation and differentiation, resulting in enhanced postnatal neovascularization [134]. In humans, standard therapeutic doses of EPO increase the number of functionally active endothelial progenitor cells not only in healthy subjects but also in patients with advanced renal failure [135]. The enhanced recruitment of endothelial progenitor cells might be of therapeutic importance since intracoronary infusion of autologous progenitor cells beneficially affects post-infarction remodeling in post-AMI patients [136].

Antioxidative – Anti-inflammatory Properties

EPO exerts an anti-inflammatory effect in cardiac myocytes and ameliorates the myocardial inflammatory response following ischemia-reperfusion (I/R), which largely

contributes to the induced myocardial dysfunction. Exposure of cultured rat cardiomyocytes to EPO prior to anoxia/reperfusion prevented the oxidant stress and the conversion of myocytes to a proinflammatory phenotype that increases polymorphonuclear neutrophil infiltration [123]. *In-vivo*, EPO prevented the increase in the I/R-induced myeloperoxidase activity, an index of polymorphonuclear neutrophil infiltration [57]. The anti-inflammatory effect of EPO is mediated by upregulation of endothelial NO synthase (eNOS), resulting in increased NO production which inhibits chemotactic-activating factors and reduces the oxidative stress. The EPO-induced upregulation of eNOS was demonstrated to be mediated by the activation-translocation of the nuclear transcription factor AP-1 (activation protein 1) via a PI3-kinase dependent signalling pathway (Fig. 3). However, it should be noted that the abovementioned beneficial anti-inflammatory effects of EPO were achieved using doses higher than those used in humans in clinical practice [137]. The anti-inflammatory profile of EPO has also been validated in studies focusing on experimental brain injury, where EPO injection into ischemic rodent brain reduced the number of inflammatory cells associated with injury in the brain tissue [90].

In conclusion, EPO administration even after the occurrence of an ischemic event can protect the viable myocardium, offering a time-window that could be of therapeutic importance in clinical practice. It should also be pointed out that the beneficial effect of EPO in several studies after only a single administered dose, before any measurable increase in hematocrit, clearly demonstrates that the cardioprotective profile of this pleiotropic cytokine is independent of hematopoiesis. Despite the profound limitations of extending conclusions derived from experimental data in similar scenarios in the clinical arena, a single dose of EPO after an ischemic cardiac episode might prove beneficial in salvaging the myocardium at risk. Nevertheless, well-designed randomized clinical trials should address the issue of EPO-mediated cardioprotection in several clinical settings such as following acute coronary syndromes or cardiac surgery.

RESISTANCE TO ERYTHROPOIETIN

Clinical experience has shown that not all patients will respond to erythropoietin. Although several studies indicate that patients with diabetes, women, and black patients on hemodialysis require a higher dose of rHuEpo than others to attain target hemoglobin levels, it seems that compelling evidence exists only for women requiring a higher dose than men, and identification of other variables, potentially modifiable, responsible for hyporesponsiveness to EPO should be sought [138]. Indeed, a small group comprising 5-10% of patients treated with EPO will show either no response or a blunted response [139]. To obtain an adequate response to r-HuEpo, the plasma concentrations of the hormone must be increased to values greater than those of normal individuals. According to the European Best Practice Guidelines (EBPG) a continued need for >300 IU/kg per week when administered subcutaneously or >400 IU/kg per week when administered intravenously is defined as an inadequate response to r-HuEpo [140]. US guidelines define hyporesponsiveness as a failure, in the presence of adequate

iron stores, to achieve and maintain the target hemoglobin level at a r-HuEpo dose of 450 IU/kg per week when administered intravenously or 300 IU/kg per week when administered subcutaneously [141].

Many factors are responsible for resistance to r-HuEpo administration. The most common causes are iron deficiency, blood loss (often occult) and infections or inflammatory conditions including malignancy [139]. Other factors include secondary hyperparathyroidism, aluminum toxicity, vitamin B12 or folate deficiency, bone marrow dysfunction, red cell enzyme defects, hemoglobinopathies, hemolysis [139], vitamin C deficiency [142], L-carnitine deficiency [143] and interactions with certain drugs such as ACE inhibitors and angiotensin II receptor blockers [144] or chemotherapeutic and immunosuppressive drugs [145].

Iron deficiency is the most common cause of initial or acquired resistance to r-HuEpo. Absolute iron deficiency is defined as a ferritin value <100 ng/ml and transferrin saturation < 20%. Functional iron deficiency is present when the demands for iron exceed its availability to meet the needs of erythropoiesis. In this situation ferritin and transferrin values are normal, so other indicators have been investigated [143]. Of these, more reliable parameters could be the percentage of hypochromic red cells [139] and the concentration of transferrin receptor in circulating blood [145]. A percentage of hypochromic red cells >10% with normal values of ferritin and/or transferrin probably shows functional iron deficiency [139]. Iron must be present in sufficient numbers in the bone marrow for normal erythropoiesis. Iron deficiency is common in hemodialysis patients because of the chronic blood loss that occurs from laboratory tests and blood remaining in the dialyzer and tubing. In addition r-HuEpo by accelerating erythropoiesis, further increases the demand of iron. The best way to replete iron stores in these patients is with intravenous iron [143]. Because there is great need for iron in the erythropoietin-stimulated erythroid progenitors, serum ferritin and transferrin saturation levels should be maintained over 300 ng/ml and 30% respectively. On the other hand, iron overload may lead to an enhanced risk for infection, cardiovascular complication and cancer, so overtreatment with iron should be avoided. A safe upper limit of serum ferritin to avoid iron overload is not clearly defined [146].

Chronic blood loss due to repeated blood sampling, occult gastrointestinal bleeding, blood losses in the dialyzer of hemodialysis patients [139] and meno-metrorrhagia lead to iron deficiency and apparently diminished response to r-HuEpo therapy. Inflammatory states are also frequently the cause of poor response to r-HuEpo. These include chronic infections, postsurgery, rheumatologic diseases, malignancies, or the dialysis process itself [143]. Resistance to r-HuEpo during these states is probably due to several mechanisms. The most frequent cause is limited iron availability for heme production from iron stores, in the endoplasmic reticulum system, in the presence of infection. Another important mechanism is increased generation of inflammatory cytokines such as IL-1 β , IL-6, IFN- γ and TNF- α [7]. These cytokines are involved in the inflammatory process as they are directly produced by macrophages [142] and might antagonize the action of r-HuEpo at a cellular level [147],

thereby causing resistance to r-HuEpo therapy. A future use of specific anti-cytokine therapy may be the treatment [148]. C-reactive protein [143] and increased plasma fibrinogen greater than 4 g/l [145] are useful diagnostic tests when inflammation is suspected.

It has been generally recognized in the past few years that uremia is a chronic inflammatory state. C-reactive protein (CRP), ferritin, fibrinogen and interleukin 6 are raised even in the absence of overt infection [149]. In patients with end stage renal disease, r-HuEpo resistance has been linked with inflammation [150].

Secondary hyperparathyroidism is a well-known consequence of renal failure, but also a cause of resistance to r-HuEpo therapy. Several potential mechanisms are responsible for this. These include a direct toxic effect of parathyroid hormone on endogenous erythropoietin synthesis and on bone marrow erythroid progenitors, as well as an indirect effect *via* the induction of bone marrow fibrosis and interference with erythropoiesis [142]. Patients who have severe hyperparathyroidism with osteitis fibrosa show considerable resistance to erythropoietin due to replacement of the cellular components of the bone marrow by fibrous tissue [139]. However, even in normal bone marrow, deficiency in calcitriol as one of the causes of hyperparathyroidism, could impair erythropoiesis, since calcitriol induces proliferation and maturation of erythroid progenitor cells [151]. In case of unexplained resistance to epoetin, investigation of secondary hyperparathyroidism is strongly recommended including serum parathyroid hormone, calcium, phosphate, alkaline phosphatase, skeletal radiology and even bone biopsy where needed [152]. Medical or surgical parathyroidectomy is effective in reducing r-HuEpo resistance [143].

Aluminum overload is another cause of r-HuEpo hyporesponsiveness in uremic patients who take high amounts of aluminum-containing compounds such as oral phosphate binders [145]. The mechanism of this effect is only partly understood, but it is generally believed to relate to the existence of interference with iron transport and utilization, inhibition of heme synthesis and increased hemolysis due to increased red cell fragility. The treatment is withdrawal of aluminum-containing phosphate binders and by intermittent deferoxamine chelation therapy [139].

Vitamin deficiency states such as folic acid or B12 deficiency can aggravate the anemia of chronic renal failure due to ineffective erythropoiesis and contribute to resistance to r-HuEpo therapy. Also, vitamin C deficiency is associated with decreased availability of stored iron. Vitamin C administration can improve iron availability by permitting better iron mobilization from the macrophage-monocyte system [142]. L-carnitine deficiency seems to contribute to refractoriness to r-HuEpo. The benefits of such supplementation are still unclear, but some studies have shown that L-carnitine may increase reticulocyte count or improve mechanical stability of erythrocytes by facilitating the uptake of structural lipids [153,154].

Bone marrow dysfunction due to any cause (myelodysplastic syndrome, aplastic anemia, marrow infiltration by tumor, advanced multiple myeloma, etc.) can also lead to

refractoriness to r-HuEpo [139]. Hemolysis, red cell enzyme defects and hemoglobinopathies like sickle cell disease and thalassemia also need high-dose EPO treatment. The reason is the accelerated destruction of red blood cells. EPO in usual doses may not be effective in correcting anemia in these individuals [155].

Finally, much interest has focused recently on the potential of two classes of drugs to suppress erythropoiesis and induce some resistance to erythropoietin therapy. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs), seem to have such an effect by mechanisms that are not yet completely elucidated. It has been known that the renin-angiotensin system is linked with the production of endogenous erythropoietin and this is the reason why patients with renal artery stenosis become polycythemic. Suppression of angiotensin II production by ACE inhibitors may inhibit erythropoietin synthesis [144]. However, hemoglobin levels can be decreased in patients stable on epoetin administration in whom an ACE inhibitor is introduced [156,157]. Several possible mechanisms have been described. Angiotensin II can stimulate erythroid progenitor cell growth *in vitro* and captopril can inhibit this [158]. Besides, ACE inhibitors have been shown to reduce production of interleukin-12, a cytokine known to stimulate erythropoiesis and also increase plasma levels of a natural stem cell regulator called Ac-SDKP which inhibits normal early progenitors [144]. However, the ability of ACE inhibitors to decrease r-HuEpo responsiveness has been debated with several small and uncontrolled studies showing conflicting results. It seems that ACE inhibitors may evoke a degree of epoetin resistance particularly at high doses, so reducing the dose to these individuals should be considered. The evidence of angiotensin II blockers producing this effect is less persuasive, due to a paucity of data.

ADVERSE EFFECTS OF RECOMBINANT ERYTHROPOIETIN

Hypertension is the most common side-effect noted with r-HuEpo administration [159-161]. It usually occurs within a few weeks to months after initiation of r-HuEpo therapy [159], and usually induces a 10 % increase in blood pressure [160]. Patients with a history of hypertension, even if they are normotensive in the anemic state, are at a higher risk for developing hypertension under r-HuEpo treatment [162]. Approximately 30-35 % of dialysis patients will develop hypertension [163]. The specific mechanism is unknown, but there are many theories based on animal and human studies. Initially it was believed that the reason for r-HuEpo-related hypertension, was the increased erythrocyte mass due to epoetin, which caused a rise in blood viscosity as well as a reversal of hypoxic vasodilatation [159]. However, studies showed that erythropoietin-treated iron-deficient patients had a similar rise in blood pressure despite persistent anemia [164]. Moreover, multiple small blood transfusions administered, failed to increase blood pressure. These observations clearly excluded the change in hematocrit and erythrocyte mass as the mediator of EPO-induced hypertension. Currently, it is believed that erythropoietin causes a vasoconstriction-dependent hypertension, due to mediating an elevation of cytoplasmic calcium concentration which leads to the

development of resistance to the vasodilatory action of nitric oxide, an increase of endothelin production, upregulation of tissue renin and angiotensinogen expression, and a possible change in vascular tissue prostaglandin production [164]. Recombinant human EPO-induced hypertension can easily be treated by initiating or increasing antihypertensive medications [143].

Hypertension and increased viscosity due to r-HuEpo treatment may cause hypertensive encephalopathy, cerebral convulsion, hypoperfusion, focal cerebral edema and seizures [165] at approximately 5% [166]. One other possible side-effect of r-HuEpo is the risk for thromboembolic events. This relates to a prothrombotic effect that r-HuEpo may have, due to changes in platelet aggregability [160] as well as the increased viscosity. However, chronic renal failure on its own predisposes to thrombotic events [167]. Controlled studies provided no evidence of a relevant increase in the risk of thromboembolic events during r-HuEpo therapy [168]. Nevertheless, targeting a high hematocrit level with EPO therapy may not be advisable; in chronic kidney disease, the Amgen Normal Hematocrit Cardiac Trial showed an increased risk of thrombotic events in the high (mean 42% vs 30%) hematocrit group [24,75,143]. The incidence of nonfatal myocardial infarctions (3.1% vs 2.3%), vascular access thrombosis (39% vs 29%), and all other thrombotic events (22% vs 18%) was higher in the group randomized to 42% hematocrit. On the other hand, in cancer patients, 6 randomized controlled studies and a meta-analysis reported the overall incidence of thromboembolic events, noting a numerically, but not statistically, higher thromboembolic risk (4-7% vs 0-6% in controls) following erythropoietin therapy [169]. Pooled results from 12 randomized, controlled trials of the meta-analysis revealed a 1.55-fold higher risk of thromboembolic events with rHuEpo therapy compared with controls [169].

The use of r-HuEpo from athletes nowadays, in order to increase their performance, may lead to fatal consequences. Hematocrits greater than 55% are associated with unacceptable risks which include encephalopathy, seizures, vascular distention, impairment of blood flow, resulting in tissue hypoxia and thromboembolic phenomena, possibly leading to pulmonary embolism, myocardial infarction or stroke [170].

A rare but serious side effect of prolonged erythropoietin treatment is pure red cell aplasia (PRCA) [171-177], which is defined as severe anemia secondary to virtual absence of red blood cell precursors in the bone marrow [173]. The disease is generated by epoetin-induced antibodies that neutralize all the exogenous r-HuEpo and cross-react with endogenous erythropoietin [172,174]. The result is ineffective erythropoiesis and undetectable serum erythropoietin levels [175]. Anti-erythropoietin antibodies are polyclonal and able to neutralize very high concentrations of the native protein [176]. Although PRCA secondary to r-HuEpo treatment is very rare, physicians should always consider it in cases of rapidly worsening anemia and resistance to treatment. The main features of the disease are severe anemia, low reticulocyte counts, normal platelet and granulocyte counts, and bone marrow smears exhibiting an almost complete absence of red cell precursors [176]. The hemoglobin

concentration declines very quickly, at a rate corresponding to a red cell life span (approximately 1 g/dl/week). When other known causes of anemia have been excluded, bone marrow evaluation and testing for erythropoietin antibodies are indicated [176]. A recent study showed that PRCA appears more frequent with epoetin alfa used in Europe (Eprex), than with epoetin alfa used in U.S.A. (Epogen) or epoetin beta (NeoRecormon) [177]. This concludes that PRCA appears to be product specific according to product formulation. However, it must be underlined that the incidence of PRCA cases has been coincident with a major shift from intravenous to subcutaneous administration of r-HuEpo [175]. In case of PRCA, treatment with r-HuEpo must be stopped immediately without switching to another type of erythropoietin, because anti-erythropoietin antibodies cross-react with other erythropoietins. Nearly half of these patients seem to respond to immunosuppressants [176].

In 15 countries surveyed, including those where cases have been reported, an estimated 800,000 renal failure patients have been treated with Eprex. As of September 15, 2001, 40 cases of confirmed or suspected PRCA had been reported from various countries in the world in patients with chronic renal failure treated with Eprex, most occurring after 1998. The overall estimated reporting rate of the event in the 15 countries appeared to be <1:10,000 in renal failure patients. According to a more recent study [171], between January 1998 and April 2004, 191 cases of epoetin-related PRCA were reported, of which 175 cases for Eprex, 11 cases for Neorecormon and 5 cases for Epogen. After reaching a peak incidence in 2001, adopting procedures ensuring appropriate storage, handling and administration of Eprex, the incidence of PRCA subsequently decreased dramatically by 83%.

Typically, following months to years after initiation of therapy, patients developed sudden worsening of anemia unresponsive to increasing doses of erythropoietin. PRCA was confirmed by bone marrow evaluation and in most cases neutralizing antibodies to erythropoietin were detected in serum. All of these patients became transfusion-dependent and did not respond to other erythropoietins when treatment was tried following the diagnosis or suspicion of PRCA. Hence, physicians were advised to monitor clinical response to Eprex. In patients developing sudden lack of efficacy, or worsening of anemia, typical causes of non-response, such as iron, folate or vitamin B12 deficiency, aluminum intoxication, infection or inflammation, blood loss and hemolysis, should be investigated. If PRCA is suspected and no cause can be identified, testing for erythropoietin antibodies and bone marrow examinations should be considered and therapy with Eprex must be discontinued immediately. Patients should not be switched to another erythropoietin. Other causes of pure red cell aplasia should be excluded, and appropriate therapy instituted. Other not so serious adverse effects of r-HuEPO administration comprise hyperkalemia, iron deficiency, prolonged duration of dialysis and an influenza-like syndrome [166,178].

CONCLUSION

The production of recombinant human erythropoietin has revolutionized the treatment of anemia associated with

chronic renal failure and several other medical conditions, including chronic heart failure, and has led to a greater understanding of anemia pathophysiology. Anemia has been shown to be independently associated with increased mortality and disease progression. Furthermore, preclinical studies have established erythropoietin to be a pleiotropic cytokine with anti-apoptotic activity and tissue-protective actions in the cardiovascular system.

The erythropoietin receptor is widely distributed in the cardiovascular system, including endothelial cells, smooth muscle cells and cardiomyocytes. Erythropoietin has potentially beneficial effects on the endothelium including anti-apoptotic, mitogenic and angiogenic activities. Other effects of erythropoietin are related to its pro-angiogenic effects on endothelial cells, which could be of potential value in patients with ischemic heart disease. These preclinical findings suggest that erythropoietin may have potential effects in cardiovascular disease beyond correction of hemoglobin levels. Apart from possible anti-inflammatory properties, erythropoietin may enhance myocyte contractility, can stimulate the development and mobilization of immature non-differentiated stem cells into ischemic areas of the myocardium, has the potential of lasting protection with significant cell repopulation of the damaged myocardial tissue, and with its pro-proliferative actions it may improve cardiac recovery following ischemic insults.

Certain caveats relate to possible side-effects of erythropoietin, including pro-thrombotic or platelet-activating effects and the development of hypertension. Furthermore, EPO treatment is relatively costly and entails some inconvenience in administration and monitoring. Due to these shortcomings, there has been ample interest in developing a gene therapy strategy, whereby single administration of the EPO gene would possibly ensure the long-term delivery of EPO [179-180]. Nevertheless, this futuristic approach notwithstanding, early studies in heart failure patients with anemia suggest that erythropoietin therapy is safe and effective in reducing left ventricular hypertrophy, enhancing exercise performance and increasing ejection fraction. On the other hand, the issue of erythropoietin resistance has also been raised. It seems that not all patients have a good response to erythropoietin therapy. Failure of therapy can be caused by iron deficiency, infection, uremia, blood loss, and secondary hyperparathyroidism. Recently, interactions with certain drugs, such as immunosuppressors, interferon, angiotensin converting enzyme (ACE) inhibitors, and angiotensin II type 1 receptor blockers (ARBs), have been reported as a cause of erythropoietin resistance, impairing the response to erythropoietin treatment. The inhibitory effect of ARBs on erythropoiesis by recombinant erythropoietin treatment was smaller than that of ACE inhibitors. When other causes of erythropoietin resistance have been ruled out, physicians should check the interaction of erythropoietin with other drugs, including ACE inhibitors and ARBs. In patients with erythropoietin resistance linked to ACE inhibitors, a change to an ARB should be considered.

Finally, the use of erythropoietin may become an increasingly attractive therapeutic modality in patients with heart failure and anemia, and due to its pleiotropic effects and multiple actions on the cells of the cardiovascular

system culminating in cardioprotection, erythropoietin may swiftly become part of our therapeutic armamentarium in patients with myocardial injury following myocardial infarction and join the arsenal of therapeutic approaches directed against other cardiovascular diseases.

ABBREVIATIONS

ACE	=	Angiotensin converting enzyme
AIDS	=	Acquired immunodeficiency syndrome
CEPO	=	Carbamylated erythropoietin
CHF	=	Congestive heart failure
CHO	=	Chinese hamster ovary (cells)
CNS	=	Central nervous system
EPO	=	Erythropoietin
ESRD	=	End stage renal disease
HIV	=	Human immunodeficiency virus
IL	=	Interleukin
LVEF	=	Left ventricular ejection fraction
LVH	=	Left ventricular hypertrophy
NYHA	=	New York Heart Association
PRCA	=	Pure red cell aplasia
TNF	=	Tumor necrosis factor
rHuEpo	=	Recombinant human erythropoietin
VO ₂ max	=	Peak oxygen consumption

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