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# Erythropoietin update 2011

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## Summary

Traditionally, erythropoietin (EPO) is described as a hematopoietic cytokine, regulating proliferation and differentiation and survival of the erythroid progenitors. The recent finding of new sites of EPO production and the wide spread distribution of EPO receptors (EPO-R) on endothelial cells, cardiomyocytes, renal cells as well as the central and peripheral nervous system raised the possibility that EPO may exert pleiotropic actions on several targets. Indeed studies (mainly preclinical) have documented protective, non-hematopoietic, abilities of EPO in a variety of tissue. However, the data obtained from clinical studies are more skeptical about these properties. This article provides a comprehensive overview of EPO and its derivatives.

**key words:** **erythropoietin • erythropoietin-receptor • erythropoiesis-stimulating agents • anemia**

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## Background

Erythropoietin (EPO) is widely used for treatment of anemia in patients with chronic kidney disease (CKD) and in cancer patients receiving chemotherapy [1,2]. The biggest advantage of EPO administration is increased quality of life (QoL). In many studies EPO has been shown to posses properties beyond its traditional role as a hematopoietic cytokine (Table 1) [1–3]. EPO may directly and indirectly affect different cells, enhance antioxidant enzyme production, antagonize the cytotoxic action of glutamate, metabolize free radicals, normalize cerebral blood flow, affect neurotransmitter release, stimulate neoangiogenesis, modify inflammation and promote endothelial progenitor cell proliferation and differentiation [1,4,5]. On the other hand over the last decade several studies have reported increased mortality and cardiovascular events when erythropoiesis-stimulating agents (ESAs) were administered to CKD patients [4]. Moreover, a number of promising outcomes mediated by EPO, in preclinical studies (*in vitro* and *in vivo*), were not confirmed in clinical studies [5,6].

This article provides an overview of EPO and its derivatives as a cardiovascular, neuroprotective or renoprotective agent. We also consider the relationship of EPO with malignancy and inflammation and discuss the common side effects associated with its administration.

## EPO and EPO-receptors

EPO was purified in 1977, the gene was cloned in 1985 and the EPO receptor was cloned in 1989 [5]. Recombinant human EPO (rHuEPO) has become a successful clinical application of recombinant DNA technology [7]. There are a few forms of rHuEPO: EPOetin-α, EPOetin-β, EPOetin-δ, a long-acting analogue darbepoetin α (the second generation) and a continuous EPO receptor activator (CERA, the third generation). In humans, EPO is produced by peritubular fibroblast-like type 1 interstitial cells located in the renal cortex and outer medulla [7]. The kidney is a major producer of EPO in adults but not the only one; 10% of the circulating EPO originates from non-renal tissue [8]. EPO is expressed in the liver, brain, spleen, lung and testis. During fetal development, the main producer of EPO is the liver. A comprehensive study indicated that a liver to kidney shift in EPO production occurs in late gestation; the molecular mechanisms underlying this shift are still obscure. Moreover hepatocytes remain the primary cells responsible for extrarenal EPO synthesis [9,10].

EPO is a 34,000-da, 165 amino acid glycoprotein that is synthesized mainly under hypoxic condition [9]. In fact, EPO is the only hematopoietic growth factor whose production is regulated by hypoxia. The induction of EPO synthesis by low oxygen levels lead to discovery a widespread system of hypoxia-inducible factors (HIFs). HIF-1 and HIF-2 are transcriptional activators each composed of an α and β subunit [11]. The α subunit is a cytoplasmic protein containing an oxygen degradation domain (ODDD) and a transactivation domain (TAD) [11]. The β subunit is located in the nucleus. HIF-3 is a likely inhibitor of EPO gene transcription. The primary effect of EPO on the red blood cell (RBC) line, especially the colony forming units-erythroid (CFU-E), is the promotion of RBC survival by protecting these cells from apoptosis [11].

EPO receptors belong to the super-family of cytokine receptors and can be located on the plasma membrane of bone marrow erythroid progenitor cells, cardiomyocytes, cardiac fibroblasts, endothelial and vascular smooth muscle cells, gastric cells, retinal and prostate cells, human hair follicles [8] and auditory hair cells in the inner ear [12–15]. EPO-R binding triggers at least 3 intracellular signaling cascades: (1) Janus tyrosine kinase 2 (JAK2) a cytoplasmic tyrosine kinase that phosphorylates tyrosine residues itself and provides docking sites for signal transducer and activator of transcription 5 (STAT5), (2) phosphatidylinositol-3 kinase (PI3K), and, (3) RAS/mitogen-activated protein kinase (MAPK) [16]. STAT5 and MAPK induce transcription of target genes involved, mainly with inhibition of apoptosis with cell proliferation. PI3K inhibits apoptosis by activating its downstream effector Akt, and the activation of Akt by EPO also induces activation by phosphorylation of endothelial nitric oxide synthase (eNOS) and prevents neointimal hyperplasia [16].

## EPO and Cardiovascular Protection

The prevalence of anemia among patients with chronic heart failure (CHF) is estimated over 20% and has a multifactorial etiology [17–19]. Anemia has been identified as a strong prognostic factor associated with poor outcomes (with worsening symptoms and increased mortality) among patients with CHF [20–22]. In a systematic review and meta-analysis of randomized trials, the authors evaluated the efficacy and safety associated with the use of ESAs for correcting anemia in patients with CHF [23]. In patients with CHF and anemia ESAs compared with control, were associated with a significant reduction in CHF-related hospitalizations (OR=0.41; 95% Confidence Intervals [CI] 0.24–0.69) [23]. Moreover, ESA treatment was associated with improved quality of life and left ventricular ejection fraction, lower brain-natriuretic peptide (BNP) levels and improved exercise tolerance test performance. However, the effect of ESAs on mortality was inconclusive (OR=0.60; 95%CI 0.51–1.42), but available date could suggest that ESAs have a favorable effect on all-cause mortality [23]. The impact of ESAs on morbidity and mortality in patients with HF has been evaluated in the post-TREAT meta-analysis [24]. The use of ESAs to treat anemia in patients with HF was associated with neutral effect on both mortality (RR=1.03; 95%CI 0.89–1.12; p=0.68) and non-fatal HF events (RR=0.95; 95%CI: 0.82–1.10; p=0.46) [24].

Patients with CKD are more at risk of cardiovascular events, particularly young dialysis patient, whose mortality is up to 100 times greater than for the general population [25–27]. In dialysis patient, cardiomyopathy predisposes to HF and death [27]. In contrast, a reduction in left ventricular mass index (LVMi) is associated with the increase in both all-cause and cardiovascular survival rate [28]. The changes in the LVMi among anemic CKD and end-stage renal disease patients treated with recombinant human erythropoietin were evaluated in a systematic review of papers published between 1990 and 2007 [28]. This meta-analysis revealed, that in patients with severe anemia, defined as mean baseline hemoglobin (Hb) <10 g/dl, a significant decrease in LVMi was observed. There was no such significant beneficial impact on LVMi in the moderate anemia group with target Hb above 12 g/dl. This study despite its limitations (e.g. potential for development of EPO-induced hypertension and

**Table 1.** Non-hematopoietic mechanism of tissue protection by erythropoietin.

| Type of tissue protection | Possible mechanisms of action |
|---|---|
| Cardioprotection | Reduces apoptosis, modifies inflammation, increases endothelial nitric oxide synthase, stimulates angiogenesis, promotes endothelial progenitor cells proliferation and differentiation, enhances antioxidant enzyme expression and reduces the rate of free radical production, improves cardiac function reflected by increased ventricular developed pressure ($dP/dt_{max}$) and relaxation ($dP/dt_{min}$), reduction of left ventricular mass index and increased ejection fraction. |
| Neuroprotection | Antagonizes glutamate's cytotoxic action, normalizes cerebral blood flow, stimulates neoangiogenesis, promotes endothelial progenitor cells proliferation and differentiation, affects neurotransmitter release, modifies inflammation and immune response, stimulates non-differentiated Schwann cells to proliferate, reduces apoptosis, enhances antioxidant enzyme expression and reduces the rate of free radical production. |
| Renoprotection | Reduction of apoptosis and inflammatory response, promotion of vascular repair, increasing the proliferation of tubular cells, enhances antioxidant enzyme expression and reduces the rate of free radical production. Possible autocrine-paracrine action of erythropoietin within the kidney mediates cytoprotection. |

lack of control group) supports EPO treatment of severe anemia in CKD patients [28].

In a Pilot Evaluation of the Long-term Effect of Combined Therapy With Intravenous Iron Sucrose and Erythropoietin in Elderly Patients With Advanced Chronic Heart Failure and Cardio-Renal Anemia Syndrome study, combined therapy with intravenous (IV) iron and rHuEPO showed an increase Hb level, reduction of N-terminal pro-B-type natriuretic peptide (NT-proBNP), improvement of functional capacity and cardiovascular hospitalization in elderly patients with advanced CHF and cardio-renal anemia syndrome with mild to moderate renal dysfunction [29].

## ESAs in Malignancy

Anemia is a frequent complication in cancer patients and it is one of the main causes of cancer-related fatigue [30,31]. According to some authors up to 40% of cancer patients are anemic at diagnosis and this frequency is increased following chemotherapy [32]. Before the era of ESAs, oncologists relied on transfusions to correct anemia and to improve QoL [32]. Furthermore, tumor responsiveness to chemotherapy and radiotherapy seems to be weakened in anemic patients [32]. The introduction ESAs offered an alternative method to transfusion. Clinical trials with ESAs have reported an improved QoL and decreased treatment-related anemia (including numbers of blood transfusions) [33]. Other studies suggested an improvement in survival outcome of cancer patients that received rHuEPO for anemia [33].

However, rHuEPO as treatment for cancer-related anemia could also be harmful. In cystic renal disease increased endogenous production of EPO is associated with a higher incidence of cancer [34]. Von Hippel-Lindau (VHL) disease is another example when loss of ability to degrade HIF that regulates EPO synthesis is responsible for a high incidence of spontaneous renal cell cancer [34–36]. Based on clinical trials rHuEPO was suspected to trigger tumor progression leading to decrease survival [37,38]. In the ENHANCE trial, patients with advanced head and neck cancer treated with radiotherapy had a higher risk for loco-regional progression when also receiving EPOetin β [38]. The BEST trial with metastatic breast cancer patients, who were receiving chemotherapy, was terminated prematurely because of a significant reduction of survival in women receiving ESAs [39]. A study of ESAs in non-small-cell lung cancer patients receiving palliative treatment was also terminated, because of unexpected worse survival in the ESAs arm [40]. Another problem was an increased risk of venous thromboembolism (VTE) in cancer patients following ESAs treatment. The association between cancer and increased risk of VTE is well established [41]. VTE could be an explanation of worse overall survival. However, a number of trials did not report increased VTE events. On the other hand, a number of clinical studies indicate better survival rate in cancer patients who receive anti-coagulants [40–43]. Another explanation is the binding of tumour cell erythropoietin receptors by ESAs which could stimulate tumour cell growth, decrease cell apoptosis or increase resistance to therapy [44–47]. The activation of EPO-R is similar to cell activation by growth receptors [44]. This activation depends on tyrosine kinase that phosphorylates tyrosine residues itself and provides docking sites for signal transducers [44]. Tyrosine kinase activity is important in many growth factor receptors and in oncogenes, and because of its ability to stimulate mitogenic potential it may play an important role in this mechanism. EPO-R or EPO-R mRNA are present on some cancer cells but do not necessarily indicate receptor functionality [44]. However, some researcher suggests that EPO-R expression like tumour size and lymphovascular invasion may act as a prognostic factor [48]. A very interesting hypothesis suggests that ESAs may directly stimulate tumour growth through activation of the coagulation cascade and subsequent stimulation of angiogenesis [46,48]. This hypothesis is supported by clinical evidence both of increased VTE rates and increased coagulability with ESAs exposure [46]. Pathological angiogenesis could be stimulated directly by EPO via recruitment endothelial progenitors cells or by the thrombosis pathways [46,49]. These processes are numerous and complicated and are an active area of research. In 2007 the American Society of Clinical Oncology/American Society of Hematology (ASCO/ASH) recommended against the use of EPO in anemic cancer

patient not receiving chemotherapy with the exception of the low-risk myelodysplastic syndrome [50].

## EPO and the Central and Peripheral Nervous System

Both EPO and its receptor are present in the central and peripheral nervous system [51]. Moreover, analysis of spinal fluid revealed a significant increase in EPO following its administration confirming that EPO crosses the blood-brain barrier [52]. *In vitro*, EPO protects nerve cells from hypoxia-induced glutamate toxicity, which is the main cause of hypoxia-induced nerve cell death [51]. Furthermore, in a multiple sclerosis animal model, the systematic administration of EPO alpha reduced the immune response and the inflammatory reaction including enhanced nerve recovery after spinal cord injury [53]. EPO, both in animal and human models, reduced the level of impairment after cerebral ischemia [53]. EPO and EPO-R play an essential role in neurogenesis, especially during embryonic neurogenesis [54]. In the peripheral nervous system, EPO is produced in the bodies and axon of normal ganglions in the rat dorsal root and an increased EPO level are seen in Schwann cells after peripheral nerve injury [55]. EPO stimulates non-differentiated Schwann cells to proliferate [55]. Experiments have shown that systemic administration of rHuEPO reduces apoptosis of dorsal root ganglion cells and contributes the recovery of mechanical allodynia following nerve injury [53]. EPO enhances antioxidant enzyme expression and reduces the rate of free radical production [53–55]. EPO-R and dismutase peroxide (SOD) are activated via the same metabolic pathways [53]. Akt activation by EPO inhibits various metabolic pathways that are related to cell death, such as those related to glycogen synthetase kinase 3β (GSK3β), Bcl-2-associated cell death promoting protein (BAD) and caspase-9 [53–56]. In a systematic review and meta-analysis of EPO in experimental stroke, 19 studies involving 346 animals for infarct size and 425 animals for neurobehavioral were evaluated [57]. EPO improved infarct size by 30.0% (95%CI: 21.3–38.8) and neurobehavioral outcome by 39.8% (95%CI: 33.7–45.9). The results are promising but when the impact of common sources of bias are considered, this efficacy falls, suggesting that the potential benefit may be overestimated [57]. The most recent clinical study reported that recombinant EPO failed to protect from damage induced by ischemic stroke [58].

## EPO and Renoprotection

The discovery of EPO-R mRNA and EPO-R in kidney suggested that EPO may act as a protective agent in acute kidney injury (AKI). EPO-R is expressed by mesangial cells, epithelial cells of the proximal tubule and distal tubule and the collecting duct [59]. In cultured renal cells, EPO signaling occurs through the JAK/STAT5 pathway and results in increase DNA synthesis and proliferation [60]. It is hypothesized that autocrine-paracrine action within the kidney mediates cytoprotection [60]. AKI induces apoptosis and inflammatory response but EPO decreases this processes by anti-apoptosis mechanisms, promotion of vascular repair and increasing the proliferation of tubular cells [61,62]. There is evidence that in ischemic AKI, renal expression of EPO is significantly decreased whereas EPO-R level stay unchanged thus a cytoprotective effect maybe only possible by administration of exogenous EPO [60]. Studies performed on rodents revealed a protective effect of EPO/ESAs in the experimental setting of ischemic, septic AKI or induced by hemorrhagic shock, cisplatin or radio contrast media [60]. Moreover, the cytoprotective effect was achievable both 30 min and 6 h post ischemic kidney injury compared with the respective control group [63]. In most of these studies EPO/ESAs had no effect on Hb concentration within the time frame of the studies [60–63]. Therefore, renoprotection may depend on mechanisms other than the hematopoietic properties of EPO-R [63].

However, in a recently published study – Early intervention with erythropoietin does not affect the outcome of acute kidney injury (the EARLYARF trial) [64], investigators performed a double-blind placebo-controlled trial to study whether early treatment (within 6 h of injury) with high-dose EPO (up to 50,000 U) could prevent the development of AKI in intensive care unit (ICU) patients. High dose of EPO did not alter the outcome of patients receiving EPO compared with placebo. There was no difference in the incidence of EPO-specific adverse events end early intervention with high-dose EPO was safe. However, this study had some limitations – a composite of 2 biomarkers (the proximal tubular brush border enzymes gamma-glutamyl transpeptidase and alkaline phosphatase) was insufficient for risk stratification in a patient population with a heterogeneous onset of AKI [64]. Therefore, further work is needed [65].

Few studies assessed the renoprotective effects of rHuEPO in CKD. The explanation could be that therapeutic efforts in CKD patients were made only to correct anemia and the putative hypoxic renal tissue damage as a result of anemia. Some results from recently published large trials in patients with CKD revealed no beneficial effect on progression CKD [4]. However, study by Gouva et al. in which rHuEPO therapy was started in CKD patients with only mild-to-moderate anemia, and the anemia was corrected only to subnormal levels over a period of 6 months [66]. The authors observed significantly reduced progression and significantly less need for renal replacement therapy in the group of rHuEPO-treated patients [66].

## EPO and Inflammation

Anemia is very common in chronic inflammatory diseases [4]. Pro-inflammatory cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1 and -6β are responsible for suppression of EPO production both *in vitro* and *in vivo* [67–71]. But EPO production in patients with cancer disease is not diminished enough to cause anemia, is not the only explanation of this mechanism [67]. Moreover IFN-γ, TNF-α, TRAIL and IL-1β are cytokines responsible for inhibition of the proliferation and differentiation of erythroid progenitor cells [71]. Therefore, a disturbance of erythropoiesis is most likely due to apoptosis induction, cell growth inhibition, EPO-R down regulation as a result of locally increased cytokines and as well due to impaired iron metabolism [72]. The role of reactive oxygen species (ROS) is even more complicated since they may either trigger or prevent hematopoietic proliferation and differentiation [72,73].

## EPO and Safety

EPO has been abused in sport, particularly in disciplines requiring an adequate supply of oxygen to muscles. The

first suspected cases of doping with EPO was in the 1980s; since then doping with EPO has been reported many times by the World Anti-Doping Agency [74]. Doping with EPO is associated with serious adverse side-effect beginning from hypertension, headaches and an increased number of thrombotic events and death [74]. Moreover, EPO withdrawal may be complicated in neocytolysis – hemolysis of young red blood cells in the presence of increased hematocrit [74]. The great safety concern was brought to the light when The Normal Hematocrit Study provided one of the first suggestions that the use of ESAs to raise Hb concentrations into the normal range could cause harm [75]. This concept was presented by large observational trials, all of them have shown an increase in mortality related to higher Hb values in kidney patients. The CHOIR has fuelled the debate on the safety of rHuEPO [76]. As a result, the Food and Drug Administration (FDA) has recommended the lowest possible dose to slowly raise the Hb concentration to the lowest level that will avoid the need for a blood transfusion [77]. However subsequent analysis of the trial results revealed that the cause of the worse outcomes was not the Hb level but the high ESA dose [77]. Solomon et al. observed that patients with a poor response to darbepoetin α who had subsequently higher doses of this drug to meet target Hb levels, as compared with those with a better response, had higher rates of the composite cardiovascular end point (adjusted HR 1.31; 95%Cl: 1.09–1.59) or death (adjusted HR 1.41; 95%CI: 1.12–1.78) [78].

## EPO and Hypertension

The most common side effect of rHuEPO therapy seems to be hypertension which may occur even in healthy subjects [74,79,80]. rHuEPO increases peripheral vascular resistance and decreases cardiac output [4,74]. All of that is caused by increases in endothelins, angiotensin, impaired vascular endothelial relaxation, altered calcium levels in vascular smooth muscle cells and the release of serotonin by platelets [81]. EPO has direct vasoconstrictor effects in isolated renal resistance vessels [82]. Production of endogenous EPO is as well regulated by the renin-angiotensin-system [83]. When angiotensin II are given to normal human subjects, plasma EPO levels increased [83]. A similar situation is observed when inhibition of angiotensin enzyme decrease plasma EPO [84,85]. Angiotensin II stimulates growth factors similar to insulin by inducing tyrosine-kinase receptors in vascular smooth muscle cells, which in turn produces an increase in vascular intimal hyperplasia [86]. EPO and angiotensin II seem to be similar to other cytokines that activate tyrosine-kinase receptor and that may be responsible for harmful effects of both hypertension and vascular disease progress. The main EPO mechanism for the raising the hematocrit is an increase in RBC mass but a decrease plasma volume also occurs [87]. However, the link between hypertension and an increase in hematocrit has not be proven with certainty, and arterial hypertension may occur independently of EPO`s hematopoietic effect [88–90].

## EPO and Thrombosis

Some trials and meta-analyses reported an increased risk of VTE following ESAs treatment. Before the use of EPO to treat anemia the incidence of deep vein thrombosis (DVT) and pulmonary embolism (PE) was rare in patients with CKD [81,91]. There is evidence suggesting that rHuEPO enhances procoagulant pathways, which can cause adverse effects and, therefore, potentially limit the clinical use of EPO [92]. rHuEPO increases platelet aggregability and may decreased proteins C and S levels [92]. In hemodialysis patients rHuEPO raises the platelet count and mean platelet volume but did not change the numbers of surface platelet receptors [93]. The platelet aggregation can be reversed by using aspirin but taking aspirin cannot prevent vascular access thrombosis in hemodialysis patients [93]. Vascular access thrombosis is associated with intimal hyperplasia and smooth muscle cell proliferation [94]. Another hypothesis for increased thrombosis in heamodialysis patients could be thrombocytosis due to the presence of iron deficiency caused by ESA [95]. This hypothesis is supported by a study where the use of IV iron reduced the risk of thromboembolic events by 40% [96]. Another possible explanation of increased adverse events is that chronic administration of EPO may enhance angiogenesis in the atherosclerotic plaque by increased vascular endothelial growth factor production with subsequent plaque rupture and acute coronary syndrome or stroke. From the clinical perspective, another possible explanation for high incidence of thrombosis could be viscosity of the blood, which leads to a risk of vascular thrombosis [97]. By taking into account blood viscosity as a main determinant of the work of the heart, and elevated blood viscosity appears to be both a strong predictor of cardiovascular disease and an important pathophysiological factor in the development of atherothrombosis [98].

## Conclusions

The role of ESAs and future indications are unclear. Tissue protection after ischemia and injury has been found in the brain, heart and kidney [99–101]. Benefits include an increased Hb to the recommended level in anemic patients with CKD or HF or both or in the palliative chemotherapy setting remains. An increase QoL has been reported almost in every study with ESAs. However, QoL was not generally designed as a specific end-point. Furthermore, analysis of the current available data shows major inhomogeneities in the tools used for assessment of QoL and in data reporting which suggest that only partial correction of anemia with EPO may improve QoL [102]. On the other hand a better correction of anemia with higher Hb target is associated with increased risk for stroke, hypertension, vascular access thrombosis compared with a lower Hb target [103].

Studies have demonstrated a decrease survival outcome of cancer patients that received rHuEPO for anemia. This caused great concern regarding patients on hemodialysis who have previous cancer diagnoses. However, we know that the doses of ESAs have more than tripled in the USA since ESAs were introduced and that cancer specific mortality rates remained stable among US hemodialysis patients between 1995 and 2005 [25]. Despite all the promising outcomes in numerous preclinical studies (*in vitro* and *in vivo*), EPO use as a neuroprotective drug failed in clinical studies. However, this does not mean that the neuroprotective abilities of EPO are wrong but it means that we have to be more critical in evaluating the future use of EPO [104–106]. To minimize possible side effect of ESAs therapy, a greater understanding of the physiology of this molecule and its receptors are required with the possible

alternative method of administration. Perhaps the new generation of ESAs (asialo EPO and carbamylated EPO), without the erythropoietic activity of EPO, while preserving its tissue protective properties, will provide better outcome in ongoing clinical trials [107,108].

**Conflict of interest**

None.

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