HIPPOKRATIA 2011, 15, 2: 109-115

REVIEW ARTICLE

Clinical use of erythropoietin in chronic kidney disease: outcomes and future prospects

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Abstract
The introduction of erythropoietin (Epo) in clinical practice, more than two decades ago, altered completely the management of patients with chronic kidney disease (CKD). The successful correction of anemia of CKD has resulted in reduction of associated morbidity and improvement of functionality, exercise tolerance, cognitive function and overall quality of life. Moreover, significant reduction of cardiovascular morbidity and mortality has occurred. Recently, large randomized clinical studies suggested that administration of Epo targeting at complete anemia correction is accompanied by significant increase of morbidity and mortality, compared to partial anemia correction. This observation has led to thorough investigation of the mechanisms of Epo actions and the possible contribution of other parameters including iron availability, comorbidities and resistance or hyporesponsiveness to Epo. In this context, it has been proposed that high doses of Epo are likely to exert toxic effects and pleiotropic systemic actions. Recognition of the extra-hematopoietic biologic actions of erythropoietin is a result of the better understanding of its interaction with Epo receptors in several tissues and organ systems, during fetal development as well as in the adult organism. More specifically, antiapoptotic, anti- inflammatory, angiogenetic and cytoprotective effects have been revealed in the kidneys, cardiovascular system, brain and retina. Until future studies are able to clarify the multiple beneficial or unfavorable effects of Epo, it is advisable to remain prudent in its administration, yet optimistic about its possible contribution in a number of pathologic conditions. Hippokratia 2011; 15 (2): 109-115

Key words: erythropoietin, anemia correction, cardiovascular, hyporesponsiveness, extra-hematopoietic actions, renoprotection, review

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The introduction of erythropoietin (Epo) in clinical practice, more than two decades ago, altered completely the management of patients with chronic kidney disease (CKD). The extensive use of Epo and its analogues (erythropoietin- stimulating agents, ESAs) for the purpose of anemia correction has succeeded in reduction of associated morbidity and improvement of functionality, exercise tolerance, cognitive function and overall quality of life. Moreover, significant reduction of cardiovascular (CV) morbidity and mortality has occurred. However, over the last years, much controversy has been raised about the possible risks of ESA therapy, after the publication of large- scale randomized studies that suggest greater risk with administration of large doses of Epo aiming to higher hemoglobin (Hb) targets. Moreover, the thorough investigation of the mechanisms of Epo actions has revealed multiple biologic effects that extend beyond its erythropoietic effect and may have a favorable or sometimes unfavorable contribution to these outcomes.

The original clinical observation that administration of ESAs successfully raised and maintained high Hb levels in patients with CKD raised the question of what is the optimal Hb target associated with lower CV risk. The first randomized controlled study designed to answer this question was Normal Hematocrit Study (NHS)[1]. It included patients on maintenance dialysis who also suffered from chronic heart failure (CHF) or ischemic heart disease and were randomized to receive epoetin alpha aiming to achieve and maintain a target hematocrit (Ht) of 42% or 30%. Primary end points were death or first nonfatal myocardial infarction (MI). The study was terminated prematurely due to the increased number of deaths observed in the high-Ht group that approached the boundary of statistical significance. Furthermore, an increased rate of vascular access thrombosis was reported in the same study group. The final results of the study were announced in 2007 and suggested that the relative risk of death or nonfatal MI between the two treatment arms failed to reach the prespecified boundary for statistical significance. However, the investigators of the study maintained the conclusion that a target Ht of 42% could not be recommended in dialysis patients. In Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) Study[2], non- dialysis patients with CKD (GFR 15-50 mL/min) were randomized in two groups receiving epoetin alpha with the aim to achieve Hb levels of 13.5 g/dL or 11.3 g/dL, respectively. Relative risk for the primary composite end point (death, stroke, MI and hos-

pitalization for CHF) was statistically significant between the two groups, although no statistical significance was observed when each end point was studied individually. Moreover, a strong trend towards increased mortality and rate of hospitalization for CHF was reported in the high Hb group, as well as an increased rate of serious adverse events such as thrombotic events. The third large randomized controlled study was Cardiovascular Risk Reduction by Early Anemia Treatment With Epoetin Beta (CREATE) Study[3], which compared the administration of epoetin beta with the aim to achieve the Hb target 13.0-15.0 g/dL or maintain a lower Hb level of 10.5-11.5 g/dL in predialysis patients (GFR 15-35 mL/min). Unlike the previous studies, CREATE failed to demonstrate statistical significance between the two study groups in the primary composite end point (which consisted of eight CV events). It did, however, report increased rates of vascular thrombosis, hypertension and progression to end- stage renal disease (ESRD) in the group aiming to complete anemia correction[4].

The outcomes of these studies came out to question the previous perception that ESAs are harmless and that higher Hb levels are beneficial and safe for patients with CKD. These results was further supported by later studies which investigated the effect of anemia correction on left ventricular mass and volume and concluded that Hb normalization does not have a beneficial effect on cardiac structure[5,6]. In this setting, in 2007 the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) Work Group updated its recommended Hb level target between 11.0 and 12.0 g/dL for all patients with CKD and issued a statement warning about greater risk of causing harm when targeting for an Hb level higher than 13.0 g/dL[7]. Moreover, taking into account the previous studies, the U. S. Food and Drug Administration expanded its safety advisories about the optimal use of ESAs in patients with CKD and malignancies[8]. Since then, many other committees and work groups have revised their recommendations, supporting the concern about the increased risk of mortality and adverse events at high Hb levels[4].

Anemia correction studies have been subjected to severe criticism and meta- analyses were subsequently published that questioned the validity of their results[9,10]. The lack of statistical significance at various end points, the smaller than expected number of adverse events observed, the difference in administered dose of ESA, as well as the heterogeneity of patients enrolled in the studies regarding CV comorbidities (especially pre-existing hypertension and history of coronary artery bypass) are only few of the areas of controversy[9]. Nevertheless, collectively, the three randomized studies suggest that setting a target Hb level of 13.0 g/dL or more is associated with increased risk of mortality and serious adverse events. One should easily reach the conclusion that the unfavorable outcomes of the studies are a mere result of the selected target, which is the high Hb level. However, a more thorough insight into the correlation between the increased CV risk and the intervention that led to this effect, which is anemia correction, should also take into account other parameters that may have been etiologically involved. These parameters include the different means that were used in order to achieve the selected target (different dose and dosage schedule of ESAs) and the presence of specific characteristics among the patients that may be responsible for their different response to treatment, therefore influencing the final outcome[4].

In this context, it has been proposed that the increased CV risk should not be attributed to the high Hb target level per se but to the use of high doses of ESAs in order to achieve it. This notion was originally supported by preliminary results of the prementioned studies, which demonstrate a clear association between adverse events and exposure to higher doses of ESAs[4,9,11]. In a post- hoc analysis of CHOIR study that evaluated the association between patient response to ESAs and mortality, both the inability to achieve Hb target and the use of high doses of ESAs were significantly associated with increased risk of mortality[12]. It should be noted that this association was observed in both treatment arms and remained after patient adjustment. On the contrary, patients of both groups who achieved their Hb target showed no increased CV risk. In the NHS trial, the highest rates of morbidity and mortality were observed in the subgroups of patients who failed to respond to administration of ESAs and intravenous iron[13]. Other observational and retrospective studies in large groups of patients also found a correlation between increased ESA dose and CV and all- cause mortality, irrespectively of the Ht level[14,15]. However, these studies were unable to support the causal relationship of their findings.

Two mechanisms have been suggested as responsible for the increased CV risk associated with administration of high doses of ESAs. According to the first, erythropoietin and its analogues are considered beneficial at normal doses; however at high doses they seem to exert toxic effects. ESA toxicity has been associated with the numerous extra- hematopoietic actions of erythropoietin and mainly with its effect on arterial pressure, vascular endothelium and the coagulation system[13]. Hypertension is a well documented effect of Epo and it is correlated with increased levels of endothelin-1, prostaglandin- 2 and thromboxane, decreased levels of nitric oxide and activation of the renin angiotensin system[16]. Hypertension is an independent CV risk factor through increase of systemic vascular resistance and attenuation of vascular damage and cardiac dysfunction. Moreover, Epo promotes activation of several proinflammatory cytokines by the endothelium, which may exacerbate cardiovascular remodeling and inflammation at high doses, thereby contributing to the increased CV risk. Epo administration also increases platelet count, reduces uremic platelet dysfunction and enhances blood coagulation through stimulation of E selectin, P selectin, von Willebrand factor and plasminogen activator inhibitor-1 production. At high doses, however, these effects produce a pro-thrombotic

state which, combined with the increased blood viscosity, is responsible for the higher rate of thrombotic complications. Therefore, these "toxic" effects of high levels of Epo can explain the greater morbidity and mortality observed in the studies[11, 17].

The second mechanism suggests that ESA therapy is ineffective in some patients due to lack of responsiveness to the erythropoietic action of Epo. These patients are more likely to experience unfavorable CV events and are at greater risk of death than other patients, when exposed to higher doses of ESAs[4]. The underlying causes of ESA hyporesponsiveness seem to be associated with inflammation and oxidative stress. Inflammation and oxidative stress are common in CKD, but can be exacerbated by other comorbidities including diabetes mellitus, infections and autoimmune disorders. Oxidative stress and inflammation are responsible for EPO-resistant anemia through several mechanisms, such as shortening of erythrocyte life span due to oxidation of their membrane phospholipids and reduction of their redox capacity, increased production of hepsidin and reduced production of transferrin and its receptors, resulting in reduced absorption and mobilization of iron. Furthermore, oxidative stress and inflammation accelerate atherosclerosis and endothelial dysfunction, further contributing to the increased CV risk[17]. Recognition of the hyporesponsive population is considered of great clinical importance, as they are unlikely to benefit from any increase in ESA dosage, whereas remain at greater risk of adverse events. The diagnosis of ESA hyporesponsiveness requires exclusion of other factors related to anemia resistance, such as iron, folic acid or vitamin B12 deficiency, aluminum toxicity, hemolysis, malignancies, pure red cell aplasia, osteitis fibrosa cystica and others (Figure 1). Moreover, recent

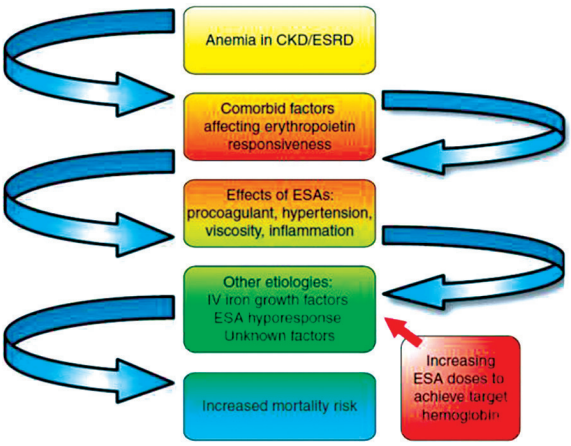

Figure 1: The interplay between factors potentially associated with the increased risk of mortality in CKD/ESRD patients targeted to higher hemoglobin levels. CKD, chronic kidney disease; ESA, erythropoietin-stimulating agent; ESRD, end-stage renal disease. Reproduced from: Rosner MH, Bolton WK. The mortality risk associated with higher hemoglobin: is the therapy to blame? Kidney Int. 2008; 74: 695-697[11].

studies that focused on management of these hyporesponsive patients suggest that intravenous iron administration successfully raises Hb levels without the need for increasing ESA doses, resulting in a lower risk of CV morbidity and mortality[4, 18].

It is obvious that the interactions between these mechanisms are far more complicated and many issues remain to be resolved in the future concerning the optimal use of Epo that combines successful management of anemia of CKD without any unfavorable systemic effects.

Extra- hematopoietic actions of erythropoietin

The pathophysiologic mechanism of Epo's hematopoietic action has been studied extensively and is well understood. Recently, the discovery of Epo receptors (Epo-R) in a variety of cells and tissues including neural cells, the female reproductive system, placenta, macrophages, cardiomyocytes, and vascular endothelial cells has suggested a potential role of Epo that extends beyond erythropoiesis. A growing body of evidence supports that Epo signaling in non- hematopoietic organs is related to tissue- protective and antiapoptotic effects[19,20].

The molecular mechanisms responsible for the various biological effects of Epo are yet to be clarified. The binding of a single Epo molecule to two adjacent Epo receptors on the surface of the target cell leads to homodimerization of the Epo-R and triggering of different intracellular signaling pathways, such as the JAK-STAT and PI3K-Akt pathway. In non- hematopoietic cells, activation of other intracellular molecules has also been revealed, including ERK1/2, MAP kinase, I-kB, protein kinase C and heat shock proteins[19]. It has been postulated that different intracellular pathways may be activated by distinct conformations of the receptor. Moreover, a single Epo-R chain can interact with other surface membrane proteins, forming heterodimeric receptor complexes. The common b- receptor subunit (bcR, CD131) has been recognized as one. Recently, the existence of two different classes of Epo receptors, with regard to affinity for Epo binding, has been revealed, suggesting that high affinity receptors mediate the hematopoietic effects whereas low affinity receptors may be involved in the extra- hematopoietic biological effects of Epo[21,22].

Cellular cultures as well as experimental studies in models of ischemia- reperfusion (I-R) injury and hypoxia have revealed cytoprotective, anti-apoptotic, anti-inflammatory and cardiovascular effects of Epo in several extra-hematopoietic tissues. Pretreatment with human recombinant Epo (hrEPO) has been shown to increase cellular survival and reduce apoptosis and infarct size in the heart, brain and kidneys. Moreover, Epo suppresses the inflammatory response to reperfusion through reduction of reactive oxygen species production and inhibition of polymorphonuclear neutrophil infiltration. Production of proinflammatory cytokines (TNF-a, IL-6) is reduced and expression of anti-inflammatory cytokine IL-10 is promoted. Systemic Epo administration promotes cardiovascular reparative processes through increased neo-

vascularization and recruitment of endothelial progenitor cells (EPCs). It has also been shown that Epo increases the levels of vascular endothelial growth factor (VEGF) and promotes the expression and phosphorylation of nitric oxide (NO) synthase in the hypoxic endothelium, resulting in increased vascular protection[19,23].

Fetal development

It has been suggested by animal model studies that the role of endogenous Epo in fetal survival and development extends beyond promotion of erythropoiesis. In the embryonic heart, Epo signaling promotes cardiac myocyte proliferation and reduces apoptosis. It is also considered essential during early neurogenesis, as it regulates neural progenitor cell differentiation and neuroprotection. In EPO or EPO-R null mouse embryos, increased neuron cell death in response to hypoxia and incomplete neural tube closure was reported[24]. These animal models also exhibited defects in physiological angiogenesis, characteristically during the second wave of blood vessel formation[20].

Nervous system

The functions of Epo in the adult nervous system have been studied mainly in cultures of neuronal cells and astrocytes, as well as in animal models of I-R injury and neurotoxicity. All have revealed increased expression of Epo-R in hypoxic neurons and a neurotrophic and cytoprotective effect of exogenous Epo, characterized by reduction of cell death and preservation of synapses. Most studies of cerebral ischemia report that hrEPO can cross the blood-brain barrier leading to reduction of infarct size and neuronal apoptosis, although this hypothesis has been questioned by others. Consistent with the ability of Epo to cross the blood-brain barrier are animal studies of brain and spinal cord trauma and subarachnoid hemorrhage, where Epo therapy improved functional recovery and was associated with preservation of myelin. The neuroprotective effects of Epo have also been shown in models of axonal injury and degeneration, as well as peripheral nerve disorders such as diabetic neuropathy and polyneuropathies. Recently, potential beneficial effects of Epo in various neuro-psychiatric disorders have been suggested by studies with Epo administration after brain ischemia, which revealed improved synaptic transmission and cognitive function and reduced brain atrophy. Epo was reported to have a beneficial effect on memory retrieval, emotional processing, mood and verbal fluency in healthy subjects, whereas small studies in schizophrenic patients reveal increased Epo penetration into the brain and improved cognitive performance after exogenous Epo therapy[20].

Cardiovascular system

The discovery of Epo receptors in myocardial and endothelial cells, as well as in vascular smooth muscle cells, implies multiple CV actions of Epo[19]. Angiogenesis is a vital procedure normally regulated by Epo/ Epo-R signaling in the developing embryo, the female reproductive system and during tissue regeneration in response to hypoxia. Recently, in has been shown that exogenous Epo administration promotes neovascularization in other hypoxic tissues and sites of vascular injury. Neovascularization occurs by angiogenesis, which refers to development of new vessels from existing ones, and vasculogenesis, which is the de novo formation of new vessels from EPCs. Epo induces EPC recruitment and increases circulating EPC levels in myocardial tissues after experimental MI. Moreover, it has been associated with increased myocardial expression of VEGF, which also promotes vascularization[25]. In models of femoral artery ligation injury and pulmonary hypertension induced by hypoxia, Epo administration was related to accelerated endothelial response, inhibited neointimal hyperplasia and increased re-endotheliazation, suggesting a protective role of Epo against vascular injury and hypoxia.

The cardioprotective effects of Epo have been studied in several experimental models of I-R injury, in which administration of hrEPO prior to or 12 hours after coronary ligation was found to reduce myocardiocyte apoptosis as well as infarct size and led to better left ventricular function recovery. Further studies of Epo-null transgenic mice subjected to coronary artery ligation and reperfusion reveal left ventricular dysfunction, disruption of vascular regeneration in the myocardial tissue, as well as increased cellular apoptosis and infarct size following ischemic injury. In human subjects following MI, increased levels of endogenous Epo were correlated to reduced infarct size, which corresponded to reduced levels of creatinine kinase[20].

A number of ongoing randomized controlled clinical studies are studying the role of hrEPO administration in patients with acute MI who are subjected to coronary revascularization (Table 1). Two of the studies compare the effect of darbepoetin alpha administration a few hours prior to or after the intervention with standard treatment only, with infarct size and left ventricular function as end points. Another pilot study compares the levels of creatinine kinase and troponine I in patients who were subjected to coronary intervention after bolus administration of 40,000 IU Epo. In Regeneration of Vital Myocardium in ST-Segment Elevation Myocardial Infarction by Erythropoietin (REVIVAL-3) Study, the effect of epoetin beta against placebo is compared in 68 patients with a first MI who were subjected to coronary angioplasty. During the 6 months of follow-up, no significant differences have been reported in LV ejection fraction and infarct size, whereas there is a slight trend of increased side effects in the Epo group. The Exogenous Erythropoietin in Acute Myocardial Infarction: New Outlook and Dose Association Study (EPAMINONDAS) is a large- scale double- blind polycentric randomized controlled phase II study with 102 patients after first MI who are subjected to coronary angioplasty. A number of endpoints are being studied at patient dismissal from hospital and during 12 months of follow-up. The preliminary results of the study have not

Table 1: Overview of randomized controlled studies investigating the effects of Epo in patients with acute myocardial infarction.

Author or acronym	Design	Patients	Dosing and type of Epo	Follow-up	Primary end-point
Lipsic et al.	Randomized controlled open-label	22 first STEMI for primary PCI	- 300 μg darbepoetin-β (~ 60,000 IU epoietin) - single IV bolus - directly before PCI	4 months	LV ejection fraction by radionuclide ventriculography
Belonje et al.	Randomized controlled open-label	466 first STEMI for primary PCI	- 60,000 IU Epo (unspecified) - single IV bolus - within 3 h of PCI	1 and ½ months	LV ejection fraction by radionuclide ventriculography
REVEAL	Randomized placebo-controlled double-blind dose-finding	210 first STEMI for primary or rescue PCI	- dose-escalation of 15, 30 or 60 x 10^3 IU epoietin-α (safety phase) - followed by efficacy phase - single IV dose	3 months	Infarct size by cardiac MR
REVIVAL-3	Randomized placebo-controlled double-blind	138 first STEMI for primary PCI	- 33,000 IU epoietin-β - triple IV bolus - after PCI and after 24 and 48 h	6 months	LV ejection fraction by cardiac MR
EPAMI-NONDAS	Randomized placebo controlled double-blind dose-finding weight adjusted	102 first STEMI after successful primary PCI	- 100 or 200 IU/kg/day of epoietin-β - triple 30 min IV infusion - within 12 h of PCI - and on next 2 days	12 months	Infarct size by CK-MB, LV ejection fraction and cardiac MR
Liem et al.	Randomized placebo-controlled	51 non-STEMI	- 40,000 IU of Epo (unspecified) - single IV dose - 8 h after cTnI elevation	72 h	CK-MB release

Main characteristics of the largest randomized controlled studies testing the effects of Epo administration in patients with acute myocardial infarction. CK, creatine-kinase; cTn, cardiac troponin; Epo, erythropoietin; IU, international units; IV, intravenous; LV, left ventricular; MR, magnetic resonance; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction, Reproduced from: Andreotti F, Agati L, Conti E, Santucci E, Rio T, Tarantino F, et al. Update on phase II studies of erythropoietin in acute myocardial infarction. Rationale and design of Exogenous erythropoietin in Acute Myocardial Infarction: New Outlook aNd Dose Association Study (EPAMINONDAS).

revealed any significant differences between study groups or any serious side effects[26].

The role of endogenous Epo or hrEPO administration in CHF appears to be even more complicated, mainly due to the presence of anemia as an independent risk factor for increased morbidity and mortality in these patients. Various studies of anemic CHF patients have revealed improved quality of life and New York Heart Association (NYHA) function class, increased LV ejection fraction and exercise tolerance, as well as reduced need for diuretics after Epo treatment. Although these clinical effects have been well associated with anemia correction, there are also implications of direct beneficial actions of Epo in cardiac functionality. Specifically, in models of CHF after acute MI without concomitant anemia, exogenous Epo administration has been correlated with improved functionality and increased angiogenesis in the myocardium, normalization of proinflammatory cytokine levels and reduction of fibrosis, suggesting a direct beneficial cardiac effect. However, these studies are limited by the development of polycythemia, hypertension and other adverse effects[20].

Renoprotective effects of Epo

As mentioned previously, Epo and its receptors are considered essential modulators of the physiologic response to hypoxia and Epo administration has been found to improve acute ischemic injury in a variety of tissues. Epo receptors have been recognized in vascular and other sites of the adult kidney, especially in tubular cells. In vitro tubular cell cultures as well as in vivo experimental studies in models of I-R injury have shown that Epo

exerts a renoprotective effect during acute kidney injury. Specifically, Epo administration triggers protective intracellular pathways that lead to increased expression of antiapoptotic genes, inhibit proapoptotic protein expression and reduce caspase activity, resulting in reduction of apoptotic cell death and increased proliferation of tubular endothelial cells[27]. Also, it has been shown that Epo- induced activation of stromal cells from the bone marrow protects tubular epithelial cells during cisplatin-induced acute kidney injury through endocrine or paracrine mechanisms[28]. In clinical practice, several aspects remain unclear with regard to the safety of Epo administration, especially concerning its dose-dependent effects on thrombogenity and hypertension[21,22].

Carbamylated Epo (cEpo) is a non-erythropoietic derivative of erythropoietin which retains the cytoprotective effect of Epo in non- hematopoietic tissues, therefore suggesting a possible therapeutic role in tubular disorders. In experimental models of tubulointerstitial injury induced by unilateral ureteral obstruction, cEpo was found to reduce epithelial apoptosis and inhibit interstitial phenotypic alterations leading to fibrosis and atrophy, through its interaction with the β- common receptor (βcR subunit), on the surface of proximal and distal tubular cells. These protective effects were not associated with any adverse effects similar to those observed after Epo administration[20,29].

Recognition of the renoprotective actions of Epo is considered even more important in the setting of CKD, mostly due to its widespread use in these patients for the purpose of anemia correction. It has been suggested that these direct renoprotective effects of Epo can delay the progress of renal disease and reduce morbidity and mortality irrespectively of complete or partial anemia correction. In this regard, large- scale, well designed studies are required in order to fully understand these direct actions and define the optimal dosage that ensures renoprotection and successful anemia correction, as well as the safety profile in order to prevent any unfavorable side effects[21,22].

The non-hematopoietic renal actions of Epo have been studied in experimental models of CKD after 5/6 nephrectomy, characterized by microvascular endothelial injury, glomerulosclerosis and ischemia- induced tubulointerstitial damage. In most of the studies, use of repeated low doses of the long-acting Epo analogue darbepoetin alpha was preferred, in order to limit any erythropoietic effects. Chronic administration of darbepoetin alpha was associated with prevention of endothelial injury, improvement of vascular damage and reduction of sclerosis in the glomeruli, as well as preservation of the architecture of the renal interstitium and peritubular capillaries. Noteworthingly, increasing doses of Epo did not exert any clinically significant renoprotective properties. The previous findings were also observed in experimental models of diabetic, toxic and immunologic kidney injury[21,30].

More specifically, the discovery of Epo receptors on the surface of podocytes has led to the assumption that Epo may have a protective effect in renal disorders characterized by podocyte injury and albuminuria, such as diabetic nephropathy and nephrotic syndrome. In an experimental model of nephrotic syndrome induced by the aminonucleoside puromycin, administration of darbepoetin alpha significantly reduced albuminuria and limited podocyte swelling and detachment of foot processes, which cause derangement of the slit diaphragms and protein leakage[31]. In another study, pretreatment with darbepoetin alpha reduced cellular apoptosis in podocyte cultures that were previously exposed to ultraviolet irradiation, but had no effect on cellular proliferation. Furthermore, chronic darbepoetin alpha administration improved glomerulosclerosis and reduced albuminuria due to prevention of podocyte apoptosis, in an in vivo study of anti- glomerular basement membrane- induced glomerulonephritis. However, the study failed to demonstrate any clinically important improvement of renal function and this finding along with the resulting polyerythremia raises serious concerns about the possible risks and benefits of Epo therapy[32].

As the pleiotropic biological effects and non- hematopoietic therapeutic applications of erythropoietin are revealed and scientific enthusiasm continues to grow, existing evidence suggesting local and systemic adverse effects should not be undermined. The level of anemia correction, hyperviscosity and thrombogenity, as a result of Epo therapy, have all been associated with increased CV and all-cause morbidity and mortality. This effect has been observed not only in patients with CKD but also in critically ill patients and patients with various malignancies subjected to chemotherapy[20]. Moreover, limited studies have demonstrated increased risk of developing retinopathy of prematurity as well as neurotoxicity after administration of high doses of Epo[20]. In this setting, future clinical trials are expected to elucidate the multiple the underlying mechanisms of erythropoietin actions, the possible risks and benefits, the specific indications and contraindications of Epo therapy, in order to define its future therapeutic use.

No conflict of interest was declared.

References

1. Besarab A, Bolton WK, Browne JK, Egrie JC, Nissenson AR, Okamoto DM, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. N Engl J Med. 1998; 339: 584-590.
2. Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, et al; CHOIR Investigators. Correction of anemia with epoetin alfa in chronic kidney disease. N Engl J Med. 2006; 355: 2085-2098.
3. Dróeke TB, Locatelli F, Clyne N, Eckardt KU, Macdougall IC, Tsakiris D, et al; CREATE Investigators. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. N Engl J Med. 2006; 355: 2071-2084.
4. Singh AK. The controversy surrounding hemoglobin and erythropoiesis stimulating agents: What should we do now? Am J Kidney Dis. 2008; 52: S5-S13.
5. Parfrey PS, Foley RN, Wittreich BH, Sullivan DJ, Zagari MJ, Frei D. Double-blind comparison of full and partial anemia cor-

rection in incident hemodialysis patients without symptomatic heart disease. J Am Soc Nephrol. 2005; 16: 2180-2189.
6. Reddan D, Lappin DW, Tierney M, Szczech LA. What have we learned about PD from recent major clinical trials? Perit Dial Int. 2007; 27: 395-399.
7. National Kidney Foundation: KDOQI clinical practice guideline and clinical practice recommendations for anemia in chronic kidney disease: 2007 Update of hemoglobin target. Am J Kidney Dis. 2007; 50: 471-530.
8. US Food and Drug Administration and Center for Drug Evaluation and Research: Information for healthcare professionals: Erythropoiesis Stimulating Agents (ESA). [Accessed: 2008 February 25]. Available from: http://www.fda.gov/cder/drug/InfoSheets/HCP/RHE2007HCP.htm..
9. Singh AK. Resolved: Targeting a higher hemoglobin is associated with greater risk in patients with CKD Anemia: Pro. J Am Soc Nephrol. 2009; 20: 1436-1443.
10. Phrommintikul A, Haas SJ, Elsik M, Krum H. Mortality and target hemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: a meta- analysis. Lancet. 2007; 369: 381-388.
11. Rosner MH, Bolton WK. The mortality risk associated with higher hemoglobin: is the therapy to blame? Kidney Int. 2008; 74: 695-697.
12. Szczech LA, Barnhart HX, Inrig JK, Reddan DN, Sapp S, Califf RM, et al. Secondary analysis of the CHOIR trial epoetin-alpha dose and achieved hemoglobin outcomes. Kidney Int. 2008; 74: 791-798.
13. Vaziri ND. Anemia and anemia correction: Surrogate markers or causes of morbidity in chronic kidney disease? Nat Rev Nephrol. 2008; 4: 436-445.
14. Regidor DL, Kopple JD, Kovesdy CP, Kilpatrick RD, McAllister CJ, Aronovitz J, et al. Associations between changes in hemoglobin and administered erythropoiesis-stimulating agent and survival in hemodialysis patients. J Am Soc Nephrol. 2006; 17: 1181-1191.
15. Zhang Y, Thamer M, Stefanik K, Kaufman J, Cotter DJ. Epoetin requirements predict mortality in hemodialysis patients. Am J Kidney Dis. 2004; 44: 866-876.
16. Krapf R, Hulter HN. Arterial hypertension induced by erythropoietin and erythropoiesis-stimulating agents (ESA). Clin J Am Soc Nephrol. 2009; 4: 470-480.
17. Vaziri ND, Zhou XJ. Potential mechanisms of adverse outcomes in trials of anemia correction with erythropoietin in chronic kidney disease. Nephrol Dial Transplant. 2009; 24: 1082-1088.
18. Coyne DW, Kapoian T, Suki W, Singh AK, Moran JE, Dahl NV, et al: DRIVE Study Group. Ferric gluconate is highly efficacious in anemic hemodialysis patients with high serum ferritin and low transferrin saturation: Results of the Dialysis Patients' Response to IV Iron with Elevated Ferritin (DRIVE) Study. J Am Soc Nephrol. 2007; 18: 975-984.
19. Cai Z, Semenza GL. Extra-hematopoietic action of erythropoietin. In: Textbook of Nephro-Endocrinology. Singh AK, Williams GH. 1st ed. San Diego, CA: Elsevier Academic Press. 2009, p.27-33.
20. Arcasoy MO. The non-hematopoietic biological effects of erythropoietin. Brit J Haematol. 2008; 141: 14-31.
21. Bahlmann FH, Kielstein JT, Haller H, Fliser D. Erythropoietin and progression of CKD. Kidney Int. 2007; 72: S21-S25.
22. Bahlmann FH, Fliser D. Erythropoietin and renoprotection. Curr Opin Nephrol Hypertens. 2009; 18: 15-20.
23. d'Uscio LV, Smith LA, Santhanam AV, Richardson D, Nath KA, Katusic ZS. Essential role of endothelial nitric oxide synthase in vascular effects of erythropoietin. Hypertension. 2007; 49: 1142-1148.
24. Chen ZY, Asavaritikrai P, Prchal JT, Noguchi CT. Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. J Biol Chem. 2007; 282: 25875-25883.
25. Nakano M, Satoh K, Fukumoto Y, Ito Y, Kagaya Y, Ishii Naoto, et al. Important role of erythropoietin receptor to promote VEGF expression and angiogenesis in peripheral ischemia in mice. Circ Res. 2007; 100: 662-669.
26. Andreotti F, Agati L, Conti E, Santucci E, Rio T, Tarantino F, et al. Update on phase II studies of erythropoietin in acute myocardial infarction. Rationale and design of Exogenous erythropoietin in Acute Myocardial Infarction: New Outlook aNd Dose Association Study (EPAMINONDAS). J Thromb Thrombolysis. 2009; 28: 489-495.
27. Breggia AC, Wojchowski DM, Himmelfarb J. JAK2/Y343/STAT5 signaling axis is required for erythropoietin-mediated protection against ischemic injury in primary renal tubular epithelial cells. Am J Physiol Renal Physiol. 2008; 295: F1689-F1695.
28. Bi B, Guo J, Marlier A, Lin SR, Cantley LG. Erythropoietin expands a stromal cell population that can mediate renoprotection. Am J Physiol Renal Physiol. 2008; 295: F1017-F1022.
29. Kitamura H, Isaka Y, Takabatake Y, Imamura R, Suzuki C, Takahara S, et al. Nonerythropoietic derivative of erythropoietin protects against tubulointerstitial injury in a unilateral ureteral obstruction model. Nephrol Dial Transplant. 2008; 23: 1521-1528.
30. Bahlmann FH, Song R, Boehm SM, Mengel M, von Wasielewski R, Lindschau C, et al. Low- dose therapy with the long- acting erythropoietin analogue darbepoetin alpha persistently activates endothelial akt and attenuates progressive organ failure. Circulation. 2004; 110: 1006-1012.
31. Eto N, Wada T, Inagi R, Takano H, Shimizu A, Kato H, et al. Podocyte protection by darbepoetin: preservation of the cytoskeleton and nephrin expression. Kidney Int. 2007; 72: 455-463.
32. Logar CM, Brinkkoetter PT, Krofft RD, Pippin JW, Shankland SJ. Darbepoetin alfa protects podocytes from apoptosis in vitro and in vivo. Kidney Int. 2007; 72: 489-49.