Potential mechanisms of adverse outcomes in trials of anemia correction with erythropoietin in chronic kidney disease

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Introduction

Advanced chronic kidney disease (CKD, stages 3–5) is almost invariably associated with anemia that is primarily caused by depressed production of erythropoietin (EPO), oxidative stress and inflammation [1,2]. This can be compounded by iron deficiency that is caused by loss of blood from repetitive laboratory tests, residual blood remaining in the hemodialysis circuits, fistula puncture site bleeding and uremic platelet dysfunction. In addition, when present, hemolytic disorders, bone marrow suppression, nutritional deficiencies, drug toxicities and hereditary diseases exacerbate the CKD-associated anemia.

The EPO-deficiency and iron-deficiency components of anemia are routinely corrected with the use of recombinant human EPO and iron preparations. However, presence of severe oxidative stress and inflammation hampers efficacy of EPO and iron in promoting erythropoiesis. In this context, severe persistent anemia despite high doses of EPO and iron is commonly due to oxidative stress and inflammation that may actually be intensified by intravenous iron and EPO administration [3–5]. Observational studies have revealed a strong association between severity of anemia and risk of morbidity and mortality from cardiovascular disease and other causes in CKD patients [6–10]. These findings have been widely interpreted as evidence for the causal role of anemia in the pathogenesis of adverse outcomes in this population. Contrary to expectations, randomized clinical trials of anemia correction revealed either no effect or increased morbidity and mortality in patients assigned to normal hemoglobin (Hb) targets [11–13]. In fact, analyses of randomized clinical trials have shown a significant increase in cardiovascular and all-cause mortality and arteriovenous access thrombosis among patients assigned to the higher than those randomized to the lower Hb targets [14–15]. These findings have been generally considered to imply that complete correction of anemia might have caused the adverse outcomes. Based on this assumption, K/DOQI guidelines were recently revised by reducing the optimum target Hb to 11–12 g/dl [16].

This article is intended to explore the reasons for the apparent contradiction between the results of observational and randomized clinical trials of anemia treatment in the CKD population. To address these issues, we have utilized relevant data derived from basic and translational studies.

Analysis of the link between anemia and adverse outcomes

As noted above, the positive correlation between severity of anemia and adverse outcomes has been widely taken to imply that adverse outcomes are caused by anemia in CKD populations. It is of note that anemia in dialysis-dependent CKD patients is generally treated with EPO and iron preparations. Consequently, when present, severe persistent anemia in this population is primarily due to EPO resistance as opposed to the lack of treatment. A frequent cause of the poorly responsive anemia in CKD patients is inflammation that is a common feature of advanced renal insufficiency [17,18] and can simultaneously cause anemia, cardiovascular disease and other disorders (Figure 1). The chronic renal failure (CRF)-induced oxidative stress and inflammation is frequently intensified by comorbid conditions such as diabetes, hypertension, autoimmune disorders and systemic and local infections (e.g. hepatitis, infected hemodialysis blood access and peritoneal catheters).

Oxidative stress and inflammation are inseparably interconnected and when present raise the risk of morbidity and mortality from cardiovascular disease and other causes by promoting endothelial dysfunction, hypertension, atherosclerosis and numerous other disorders. For instance, oxidative stress and inflammation drive atherosclerosis by promoting oxidation of lipids/lipoproteins, activation of endothelial cells, adhesion, infiltration and transformation...
Erythropoietic Impact

were taken to imply that correction of anemia can be harm-
to normal Hb groups showed either no benefit or increased
correction of anemia may improve cardiovascular outcomes
randomized clinical trials testing the hypothesis that cor-
or adverse outcomes prompted a number of
Consequently, increased morbidity and mortal-
the observation that a subgroup of patients whose Hb could
be normalized did considerably better [11] and that CKD
(p.e. polycystic kidney disease) who maintain nor-
mal Hb without EPO therapy usually do as well as or bet-
ter than their anemic counterparts [23]. It is of note that
in the randomized clinical trials of anemia correction, the
median dose of EPO administered to patients assigned to
the normal or near-normal Hb groups was two-threefolds
greater than that used in those assigned to the lower Hb
groups [11–13]. Moreover, secondary analysis of data in
the CHOIR study revealed that the inability to achieve a
target Hb and high EPO dose were each significantly as-
sociated with an increased risk of the primary endpoints,
i.e. death, myocardial infarction, congestive heart failure or
stroke [24]. Taken together, these observations point to a
possible role of non-erythropoietic actions of high doses of
EPO (and possibly iron) that are briefly described here.

Non-erythropoietic effects of EPO

EPO receptors are widely expressed in many non-
erythropoietic cells and tissues including endothelial cells,
vascular smooth muscle cells, cardiomyocytes, skeletal my-
oblasts, neurons, liver stromal cells, macrophages, kidney,
retina, placenta and a variety of cancer cells. Activation of
these receptors by EPO can account for its numerous non-
erythropoietic effects including angiogenic, anti-apoptotic,
vaso-regulatory, hemostatic and other actions that are highly
advantageous at physiological and potentially harmful at
high EPO levels/doses. Some of the positive and negative
EPO actions affecting the cardiovascular system, blood co-
agulation and kidney are briefly described here and sum-
marized in Figure 2.

Effect of EPO on arterial pressure. EPO administration
causes hypertension in CKD patients and experimental an-
imals [25,26]. The EPO-induced hypertension is unrelated
to its erythropoietic action since it occurs in both iron-
deficient and iron-sufficient CKD animals despite persist-
tent anemia in the former [27,28]. Moreover, anemia cor-
rection with multiple red cell transfusions in CKD animals
or iron repletion in iron-deficient CKD patients does not
raise arterial pressure [27–29]. These observations provide
irrefutable evidence that EPO-induced hypertension is not
mediated by changes in erythrocyte mass and instead is
caused by the drug itself. The mechanisms of action of EPO
on arterial pressure and the cardiovascular system have been

Anemia correction versus drug toxicity as the cause of adverse outcomes

The observational studies implying a link between severity of anemia and adverse outcomes prompted a number of
randomized clinical trials testing the hypothesis that cor-
rection of anemia may improve cardiovascular outcomes
[11–13]. Contrary to the expectation, patients randomized
to normal Hb groups showed either no benefit or increased
adverse cardiovascular and other outcomes. These findings
were taken to imply that correction of anemia can be harm-
ful in CKD patients. It is of note that despite large doses
of EPO and iron, only a minority of patients assigned to
the high-Hb groups reached the expected target (21% in
CHOIR and 38% in CREATE) [12,13]. This indicates that
a large segment of populations enrolled in these studies had
severe treatment-resistant anemia most likely due to sig-
nificant oxidative stress and inflammation. In addition to
their erythropoietic actions, EPO and iron have many other
effects that are beneficial at physiological and hazardous at
high levels.
Fig. 2. Figure illustrating the non-erythropoietic actions of EPO that can potentially contribute to the development of hypertension, hemodialysis blood access stenosis, diabetic proliferative retinopathy, vascular remodeling, thrombosis and tumor growth. Abbreviations: VSMC, vascular smooth muscle cell; RAS, rennin angiotensin system; EC, endothelial cells; ADMA, asymmetrical dimethylarginine; NO, nitric oxide; vWF, von Willibrand factor; PAI-1, plasminogen activator inhibitor.

Effect of EPO on the platelet and coagulation system. EPO administration significantly increases platelet count (independently of its effect on hematocrit) in ESRD patients with platelet counts <150 000/mm³ [52]. This phenomenon is due to amplification of the thrombopoietic action of thrombopoietin by EPO. In addition, by increasing intracellular calcium stores and intensifying the surge in cytosolic [Ca²⁺], EPO enhances platelet reactivity that can cause a pro-thrombotic state. Moreover, EPO can enhance blood coagulation by stimulating production of E selectin, P selectin, von Willebrand factor (vWF) and plasminogen activator inhibitor-1 and by activating pathways involved in tissue factor expression [54–57].

Thus, while the ability of EPO to reverse uremic platelet dysfunction is beneficial, high doses of EPO can cause thrombotic complications as reported in CKD and oncology patients [14,58].

Effect of EPO on the nitric oxide pathway. Asymmetrical dimethylarginine (ADMA) is a potent endogenous inhibitor of NO synthase. Elevation of ADMA is associated with endothelial dysfunction, oxidative stress and atherosclerosis. ADMA is degraded by the enzyme, dimethylarginine dimethylaminohydrolase (DDAH). EPO dose-dependently raises ADMA level via down-regulation of DDAH, lowers NO production, increases ROS generation [59] and down-regulates NO synthase expression [60] in cultured endothelial cells.

Thus, at high doses, EPO can potentially limit endothelium-derived NO production that can, in turn, contribute to endothelial dysfunction, hypertension, cardiovascular remodeling and thrombosis.
Effect of EPO on the heart. Left ventricular hypertrophy (LVH), ischemic heart disease and congestive heart failure (CHF) are highly prevalent among CKD patients. Population studies have found strong correlations between severity of anemia and prevalence of LVH and CHF in this population [61–63]. These cardiac abnormalities have been, in part, attributed to the reduction of oxygen delivery, increased cardiac output and heightened sympathetic activity occasioned by severe anemia. Several uncontrolled studies suggested that partial correction of anemia with EPO therapy may result in prevention or regression of CHF [64,65] and LVH [66–68]. However, large controlled randomized clinical trials have shown no improvement in either LVH or CHF with anemia correction in either dialysis-dependent or as yet dialysis-independent CKD patients [11,12,69–72]. It is of note that EPO has been shown to reduce the extent of apoptosis of cardiomyocytes, severity of infarct and subsequent left ventricular dilation and dysfunction following ischemia–reperfusion injury in experimental animals [73–77]. These beneficial effects are related to the direct anti-apoptotic action of EPO and are unrelated to anemia correction and as such may not be extended to chronic heart disease. Moreover, via its pro-thrombotic actions, EPO may extend coronary thrombosis and as such may be deleterious in acute myocardial infarction.

Effect of EPO on the kidney. Both vascular and non-vascular components of the kidney express functional EPO receptors [78]. Activation of the EPO receptor deters apoptosis and enhances cell survival. Several studies have shown that EPO administration can reduce apoptotic cell death and enhance recovery of kidney function and structure in various models of acute kidney injury induced by ischemia–reperfusion or nephrotoxic agents [47,79–81]. Similarly, EPO has been shown to exert anti-apoptotic action in cultured podocytes in vitro [82].

The favorable effect of EPO in promoting cell survival in models of acute injury is countered by its ability to increase platelet production, augment platelet reactivity and up-regulate endothelial cell expression of tissue factor and other pro-thrombotic molecules described earlier. The latter events can intensify injury and impede recovery by facilitating microvascular thrombosis as well as the release of pro-fibrotic, pro-inflammatory mediators from activated platelets and coagulation proteins such as thrombin. This scenario is particularly likely when parenchymal injury is accompanied by endothelial damage and dysfunction that is commonly present in many forms of acute and chronic kidney disease, hypertension and cardiovascular disorders, among others.

It has been speculated that the inherent limitation of oxygen delivery in the anemic state can accelerate apoptotic cell death and promote fibrosis and hence, contribute to progression of chronic diseases of the kidney or other organs. If true, correction of anemia should confer benefit by retarding progression of the disease. Interestingly, a few studies have shown that administration of low sub-erythropoietic doses of EPO may retard progression of renal disease in rats with renal mass reduction [83] and in diabetic db/db mice [84]. Similarly, partial amelioration of anemia with low doses of EPO was reported to slow the rate of progression to ESRD in a group of CKD patients [85]. In contrast, high doses of EPO have been consistently shown to accelerate progression of renal disease in animals with renal mass reduction [37,83,86], diabetes [84] and anti-basement membrane antibody-induced glomerulonephritis [82]. Similarly, recent large randomized clinical trials revealed a significant trend for progression to ESRD necessitating renal replacement therapy among patients assigned to the high-Hb groups [12,13]. The contribution of high levels of EPO to progression of renal disease is enforced by the recent demonstration of a strong link between development of severe diabetic proliferative retinopathy and nephropathy with a polymorphism of the EPO gene promoter that causes increased EPO production [87].

Thus, while physiologic levels of EPO confer vital benefits through its numerous non-erythropoietic and erythropoietic actions, data derived from experimental animals and randomized clinical trials suggest that high doses of EPO may accelerate progression of chronic renal disease. These effects are most likely related to the pleotropic actions of EPO amplified by pre-existing inflammation and endothelial dysfunction.

Potential contribution of non-erythropoietic actions of EPO to adverse outcomes

The unintended effects of high doses of EPO that were briefly noted above can contribute to the adverse outcomes seen in clinical trials of anemia correction in CKD populations. It is of note that resistance to erythropoietic actions of EPO is not necessarily accompanied by resistance to its non-erythropoietic effects. This is clearly exemplified by the ability of recombinant EPO to cause an equally severe hypertension and to amplify calcium signaling in iron-deficient and iron-sufficient CKD rats despite persistent anemia in the former and its corrections in the latter [26,27]. Thus, dose escalation of EPO can lead to adverse outcomes emanating from its non-erythropoietic effects in patients with EPO-resistant anemia. It is of note that CKD results in marked shortening of the erythrocyte lifespan that is not affected by the currently available therapeutic tools. Consequently, maintenance of normal erythrocyte mass in patients with advanced CKD, particularly when accompanied by severe oxidative stress and inflammation, demands high levels of sustained erythropoiesis that are far greater than that required in normal individuals. Therefore, aggressive interventions to maintain normal or near-normal erythrocyte mass in such individuals often requires high doses of EPO that can lead to drug overdose and toxicity.

Conclusions

We believe that contrary to common perceptions, association of severity of anemia with adverse outcomes shown in observational studies represents surrogacy as opposed to causal relationship. Instead, the real culprits are oxidative stress, inflammation and diminished biological capacity that simultaneously cause treatment-resistant anemia and adverse cardiovascular and other outcomes. Similarly, we
believe that increased morbidity and mortality observed in randomized clinical trials of anemia correction most likely represent drug overdose/toxicity as opposed to normalization of Hb. Consequently, in our opinion caution should be exercised when escalating the EPO dosage in an attempt to achieve the desired Hb level in EPO-resistant patients.

Conflict of interest statement. None declared.

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Epigenetic and microRNA-mediated regulation in diabetes

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Introduction

The increase in adult type diabetes is becoming a global epidemic and calls for swift actions to better understand the disease mechanisms, thus leading to improved targeted therapies. Emerging knowledge surrounding the role of microRNAs (miRNAs) in the regulation of post-transcriptional protein expression has dramatically altered the view of how target genes are regulated and how they are involved also in controlling glucose homeostasis. In addition to an improved understanding of miRNA functions, epigenetic control mechanisms are becoming better known. Thus, for example the effect of prenatal nutritional deficiencies and hereditary epigenetic changes, including DNA methylation and histone modifications are emerging as important players in the finely tuned balance of factors ultimately yielding the altered functions under various pathologic conditions.

In this article, we review the current understanding of the major epigenetic and post-transcriptional regulatory mechanisms with particular emphasis on podocytes during their injury associated with diabetic kidney damage. It is foreseen that this research line will bring major advances in diagnostics and understanding of pathomechanisms of both type 1 and 2 diabetes and will lead to identification of novel biomarkers and therapeutic targets.

Epigenetic regulation

The term epigenetics is typically defined as heritable changes in gene expression that are not encoded directly within the DNA sequence of genes. Epigenetic changes are crucial for the development and differentiation of the various cell types in an organism. However, epigenetic states can become disrupted by environmental influences or during ageing, and the importance of epigenetic changes in the development of cancer and other diseases is increasingly being discovered.

Eukaryotic genomes are packaged in two general varieties of chromatin: gene-rich euchromatin and genetically inactive heterochromatin. Heterochromatin is a tightly packed form of DNA, and its major characteristic is that DNA transcription is limited. Centromeres and telomeres are both heterochromatic (Figure 1). The euchromatin, in contrast, contains ‘active’ chromatin: DNA sequences that are being transcribed into RNA [1]. Heterochromatin replicates in the S phase (synthesis phase) of the cell cycle later than euchromatin, most likely preserving DNA structure during replication. Heterochromatin also maintains a compact and visible structure during mitosis therefore differing from euchromatin, which undergoes a typical cycle of condensation and unravelling during this process [2].

The DNA packaging densities of these two chromatin types vary along the length of the chromosome. High-density heterochromatin regions surround the centromeric region of the chromosome and have a low amount of...