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Reviewing Traditional and Novel Approaches to Managing Anemia of Chronic Kidney Disease

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Abstract:

Anemia is a common complication of chronic kidney disease (CKD). If not managed appropriately, anemia can be highly debilitating and potentially life-threatening for CKD patients. Various treatments have been developed to alleviate CKD-associated anemia, with the main goals of restoring circulating iron, promoting erythropoiesis, and downregulating hepcidin. Mainstream treatment options include iron supplements, erythropoiesis-stimulating agents (ESAs), hepcidin antagonists, and blood transfusion. However, the therapeutic efficacy of current CKD anemia treatments is hindered by extensive adverse effects, most notably iron overload and ESA resistance. HIF-PH (hypoxia-induced factor prolyl hydroxylase) inhibitors are a novel class of orally bioavailable drugs for CKD anemia. HIF-PH inhibitors adopt a distinct mechanism, stimulating erythropoiesis via transcriptional regulation of the hypoxia-activated erythropoietin (EPO) gene. This review summarizes existing and emerging therapeutic strategies for CKD-associated anemia, with particular emphasis on HIF-PH inhibitors and their clinical potential and risks.

Keywords: anemia, chronic kidney disease, erythropoietin, iron homeostasis, erythropoiesis-stimulating agents, hypoxia-inducible factor prolyl hydroxylase inhibitors

Introduction

Chronic kidney disease (CKD) features the progressive deterioration of kidney functions. A complication of CKD is anemia, where the levels of red blood cells (RBC) and hemoglobin (Hb) are below normal. Anemia becomes increasingly prevalent as kidney functions worsen, making it a major concern in therapeutic development and administration in advanced-stage CKD. Common causes of CKD-associated anemia are reduced capacity of the kidneys to produce erythropoietin (EPO), insufficient iron intake due to gastrointestinal comorbidities or hepcidin excess that limit the amount of iron available for circulation, as well as blood loss during hemodialysis. Current mainstream treatments of anemia of CKD include iron supplements delivered either orally or intravenously to replenish iron levels for the synthe-

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sis of hemoglobin (Hb); erythropoiesis-stimulating agents (ESAs) that activate genetic or biomolecular pathways leading to EPO production; hepcidin antagonists that lower hepcidin levels by interfering with the synthetic routes of hepcidin or by restricting its activity via sequestration. For patients with later-stage CKD who are on chronic dialysis, blood transfusions are often necessary to restore red blood cells (RBCs) and Hb levels.

The combined use of iron supplements and ESAs is a dominant measure for anemia correction, yet a major challenge for physicians and formulation scientists is to maintain the therapeutic effect within an ideal range and address the accumulating adverse effects when multiple treatments are applied simultaneously. CKD patients receiving the current anti-anemic treatments are at risk of iron overload, ESA resistance, infusion-related allergic or inflammatory responses and a multitude of other hardships that hinder the correction of anemia along CKD progression. Therefore, the discovery and development of novel targets and correction methods against anemia of CKD are at the forefront of hematological research.

An emerging class of drugs, HIF-PH (hypoxia-induced factor prolyl hydroxylase) inhibitors, is on a hotspot of anemia correction. Instead of relying on periodical replenishment of iron and RBCs, these agents are designed to reinforce the body's ability to synthesise EPO from a genetic level. HIF-PH inhibitors are novel ESAs that upregulate the transcription of the EPO gene and hence the expression of EPO by stimulating the oxygen-sensing HIF-EPO pathway. Despite their advantages over traditional approaches, the primary obstacle for HIF-PHIs to be a first-line treatment of CKD anemia is elevated tumorigenesis upon HIF activation.

Pathophysiology of Anemia of CKD

Anemia in CKD is primarily driven by several interrelated pathophysiological mechanisms. The kidneys are a crucial organ in secreting erythropoietin (EPO), a hormone stimulating erythrocyte production in the bone marrow. Although elevated EPO production is seen in response to anemia, in patients with renal failure, this spontaneous increase in EPO levels is feeble in correcting anemic symptoms, reflecting relative EPO deficiency as contrasted to anemic patients with normal renal function.[1]

Apart from low EPO levels, CKD patients suffer from true and functional iron deficiencies. Iron is a core element constituting hemoglobin (Hb), the functional unit of erythrocytes. Hemodialysis is a common approach to managing later stages of CKD, where blood is filtered outside the body as a replacement of the essential functions of the kidneys. Patients regularly on hemodialysis are prone to iron loss due to trapping of blood in the extracorporeal circuit, uremia-associated platelet dysfunction[2] as well as upper gastrointestinal bleeding.[3] In addition, CKD patients tend to express less serum transferrin, an important iron transporter dictating the total iron binding capacity of serum.[4]

Another factor that further contributes to iron deficiency anemia in CKD patients is excess hepcidin. Primarily synthesised in the liver, hepcidin facilitates the internalization, ubiquitination and degradation of ferroportin, which exports iron from enterocytes, hepatocytes and macrophages into the bloodstream. CKD is often associated with persistent inflammation, leading to elevated pro-inflammatory cytokines such as interleukin-6 (IL-6) and activin B. Acting synergically via the signal transducers and activators of transcription 3 (STAT3) and bone morphogenetic protein (BMP)/ small mothers against decapentaplegic (SMAD) signalling mechanisms,[5] these cytokines upregulate hepcidin expression. Diminished renal clearance further contributes to hepcidin excess. Consequently, dietary iron absorption is impeded and the amount of circulating iron sequestering iron is curtailed through sequestration within macrophages and hepatocytes.

CKD affects about 14% of adults in the United States in 2023.[6] According to data from the National Health and Nutrition Examination Survey (NHANES), anemia is twice as prevalent in CKD patients (15.4%) than the general population (7.6%).[7] Incidence of anemia surges with the progression of CKD, from 8.4% in stage 1 to 53.4% in stage 5 CKD patients. Regarding comorbidities, anemic CKD patients confront increased risk of cardiovascular disease, cognitive impairment, hospitalization and mortality rate. Given the marked prevalence and burden of treatment of anemia in individuals with CKD, there is a pressing need for strategic management of the multifaceted pathophysiology of CKD anemia, improving patients' quality of life. The next section will introduce several popularized treatments and discuss their benefits and limitations.

Conventional Treatment Options of CKD Anemia

Erythropoiesis-stimulating agents (ESAs) correct anemia through a range of distinct mechanisms. Recombinant EPO analogues mimic the action of erythropoietin, the endogenous ligand of erythropoietin receptors (EPOR) on erythroid progenitor cells in the bone marrow. Upon activation, EPOR promotes erythrocyte production by preventing apoptosis of erythroid progenitor cells.[8] Biosimilars of recombinant EPO are increasingly introduced

to the market. They exhibit comparable safety, efficacy and quality profiles as their reference medicines, but are less expensive and enable vast patient access.[9] Other ESAs act by modulating EPOR activity and enhancing their responsiveness to endogenous erythropoietin. Pharmacokinetic and pharmacodynamic modifications[10] are applied to novel drugs based on existing ESAs. For instance, epoetin alfa was processed with polysialic acid derived from *Escherichia coli* to extend the elimination half-life and reduce the dosing frequency to monthly, thereby improving dose-effectiveness.[11] ESAs are commonly administered subcutaneously or intravenously; and during dialysis as a complementation. Apart from anemia correction, ESAs have been proposed to delay CKD progression via EPOR-mediated reduction in renal cell apoptosis. However, a recent clinical study revealed conflicting results on the renoprotective effect of traditional ESAs.[12] In addition, a portion of patients experience ESA hyporesponsiveness, no effective normalization of Hb levels is seen after standard ESA dosing.[13] Since ESAs raise hematocrit, higher doses could increase blood viscosity and workload of the heart, subjecting patients to cardiovascular morbidities such as myocardial infarction and thrombosis. In chronically anemic patients, sustained use of ESAs might progressively deplete the body's iron store for erythropoiesis. Meanwhile, EPO/EPOR-induced erythropoiesis can indirectly mediate hepcidin translation,[6] necessitating co-administration of iron to sustain erythrocyte synthesis.

Iron supplementation is a parallel constituent to ESAs in the partial correction of anemia in end-stage renal disease (ESRD). Iron is typically given intravenously during dialysis sessions to provide immediate repletion of patients' iron pool. Oral iron may also be prescribed to patients with a low dietary iron intake, but to a lesser extent overall. The many restrictions of oral iron, including but not limited to poor absorption and low bioavailability; gastrointestinal adverse responses and local oxidative stress, make IV infusion the preferred route for iron therapy.[14] The use of iron supplementation is usually combined with ESAs to improve their effectiveness. Nonetheless, among hemodialysis patients regularly treated with IV iron products, a higher risk of iron overload is reported, where the iron suppled may exceed the patients' ongoing blood losses. In CKD anemia, a major concern of iron overload is the resultant elevation of plasma hepcidin levels to reduce circulating iron. Hepcidin is found to induce ischemic cardiovascular complications and atherosclerosis through iron deposition in macrophages in the vessel wall.[15] Infusion-related responses are another issue associated with IV iron. Some patients may suffer increasingly severe allergic responses from type I hypersensitivity, following repeated exposure to certain additives in the iron formulation. Hypersensitivity reactions also possibly contribute to complement activation-related pseudoallergy (CARPA), where the patient response arises from the first exposure and gradually weakens in future dosages. Activation of the complement system can cause flushing, rash and fever via mediators like complement component 5a (C5a) and C3a.[16]

While ESAs and iron supplementation complement each other and form the backbone of anemia management, physicians face difficulty in designing easy-to-follow and efficacious regimens combining the two. Therapeutic effect is restrained at submaximal levels to compromise for the accumulation of adverse effects. The current methods await future research addressing insufficient response to ESAs, iron overload, worsening of hepcidin excess, infusion-related responses and detriments to the cardiovascular system. Considering the intractable drawbacks in the conventional treatments of CKD anemia, there is an urgent need for novel approaches to alleviate anemic symptoms and potentially slow down CKD progression.

HIF-PHIs as an Emerging Anti-Anemia Medication

A key focus of future research may be the pathological transition of kidney capillary pericytes into myofibroblasts, usually triggered by kidney injury and fibrosis. This progressive loss of functional pericytes in CKD is a chief culprit of epigenic silencing of the EPO gene in the kidneys. Therefore, targeting transcription factors and associated enzymes of the EPO gene to upregulate EPO expression could be an alternative to traditional anemia treatments. Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) are a new class of drugs under intense pharmacological and clinical investigation. Through HIF stabilization, these agents are developed to correct anemia at the mRNA level, ultimately promoting transcription of the EPO gene and hence increasing EPO synthesis.[17] HIF-PHIs mimic hypoxic conditions where PH is inactivated and fail to hydroxylate HIF-α for ubiquitination and proteolytic degradation. The dehydroxylated HIF-α translocates to the nucleus, forms an active heterodimer with HIF-β, which binds to hypoxic response elements (HRE) on the EPO gene and facilitates transcriptional activation. PHD2 was found to be the main proline hydroxylase regulating the proteolysis of HIF-α.[18] A successful drug candidate will possess high selectivity for PHD2, with excellent region specificity in kidney pericytes and transdifferentiated myofibroblasts.

Roxadustat is an approved oral HIF-PHI. Clinical trials

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suggest significant reduction in hepcidin levels for non-dialysis patients. In comparison to traditional ESAs which might trigger true iron deficiency, Roxadustat exhibits stabilization or elevation in serum iron and total iron-binding capacity across all subjects. An increase in transferrin was also observed in the absence of IV iron[19], revealing the prospect of oral HIF-PHIs to replace intravenous or subcutaneous ESAs and iron supplementation as a first-line measure for correcting anemia of chronic diseases. Molidustat, a similar orally bioavailable HIF-PHI, has progressed into phase 3 clinical trials. It demonstrated high tolerance and prolonged stabilization of Hb levels within the desired range in non-dialysis patients.[21] A meta-analysis of HIF-PHIs and ESAs in non-dialysis CKD anemia patients showed no significant differences in the occurrence of cardiac and renal adverse events associated with the two classes of drugs.[20] The exact impact of HIF-PHIs on heart health is unclear and more evidence is required to assess their purported reduction of cardiovascular risks.[22]

Challenges with HIF-PHIs and Suggested Improvements

Despite the convincing efficacy and ease of administration of HIF-PHIs, a major health concern needs to be addressed before their assured approval to be widely used in anemic CKD patients. Promotion of tumorigenesis precludes HIF-PHIs from substituting the mainstream iron supplementation and ESA treatments. In addition to upregulation of erythropoiesis, HIFs are involved in angiogenesis owing to their synergic stimulation of the gene encoding vascular endothelial growth factor (VEGF).[23] The rapid proliferation and expansion of tumor cells generates a hypoxic microenvironment in which HIFs are stabilized for transcriptional activation. Enhancement of the oxygen-sensing ability of HIFs is by PH inhibitors can accelerate vasculature formation around cancer cells. Therefore, long-term use of HIF-PHIs could elevate tumor aggressiveness and metastatic potential.[24] Hypoxia is an important factor of the pathogenesis and progression of CKD.[25] Prolonged inflammation and accumulation of uremic toxins enervate immune responses against carcinogenesis, rendering CKD patients more susceptible to undesirable consequences of HIF-mediated angiogenesis. Furthermore, an activating mutation of HIF-2α has been identified in some patients with pulmonary hypertension[26], implying risk of pulmonary artery smooth muscle cell proliferation and vascular remodelling[27] upon continuous HIF stabilization.

Several measures are proposed to address the tumorigenesis effect of HIF-PHIs. The use of HIF-PHIs should be restricted in cancer patients with anemia of chronic diseases. For patients with high disposition of cancer development, existing HIF-PHIs may be administered at low dosage and combined with anti-VEGR agents to suppress angiogenesis and reduce tumor malignancy.[28] HIF-dependent programmed death-ligand 1 (PD-L1) blockers may also be investigated. HIF activation upregulates expression of CD274, the gene for PD-L1.[29] Disruption of PD-L1/PD-1 interaction can reactivate T cells for immune detection and anti-tumor responses. Lastly, because of the similar affinity of current HIF-PHIs for different subtypes of prolyl hydroxylases, they tend to initiate unwanted responses other than promoting erythropoiesis. The three members of the prolyl hydroxylase domain family, PHD1, PHD2 and PHD3, modulate transcription of various genes to different degrees, primarily including EPO, VEGF, and glucose transporter 1 (GLUT-1). For example, PHD2 (EGLN1) is the most potent in regulating HIF-1α hydroxylation; PHD3 (EGLN3) inhibits HIF-2α more effectively.[30] To discern the specificity of each PHD subtype, more comprehensive genetic studies need to be performed. The selectivity of future HIF-PHIs for one PHD would arise from precise modification of the respective gene confined to the desired location. CRISPR/Cas 9 may satisfy such specifications. For instance, a unique sequence of the EGLN1 gene is identified to design a complementary guide RNA (gRNA). Tissue-specific promoters may be used to deliver the gRNA-Cas 9 complex to kidney pericytes, where the complex recognizes the target DNA sequence upstream of a protospacer adjacent motif (PAM) sequence. Cas 9 then induces a double-strand break in the DNA, which is subsequently fixed by the cell's natural repair mechanism, leaving a mutation in the target sequence and hopefully dysfunction of the EGLN1 gene.

Conclusion

Given its prevalence and persistence, anemia remains a significant challenge in the management of chronic kidney disease (CKD), substantially affecting patients' quality of life and contributing to higher mortality rates. CKD-related anemia features a complex pathophysiology, involving an interplay of inadequate erythropoietin (EPO) production, iron deficiency, excess hepcidin and adverse events from hemodialysis. ESAs and iron supplementation constitute the backbone of modern anemia correction. While ESAs promote EPO synthesis and are hypothesized to be reno-protective, they are associated with worsening of iron deficiency and hyporesponsiveness in some patients. Consequently, iron is commonly supplied alongside ESAs, yet can bring the risk of iron overload, hepcidin elevation and thus unbalanced iron homeostasis upon chronic use. Simultaneous use of multiple treatment regi-

mens amalgamates their side effects. Both ESAs and iron are typically administered intravenously, presenting difficulty in patient compliance and possible infusion-related reactions. This highlights the need for novel drug classes and more convenient delivery methods. Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) are emerging as promising alternatives, offering abundant oral bioavailability and stabilization of plasma iron and hemoglobin levels. While they address the epigenetic silencing of the EPO gene in transdifferentiated myofibroblasts of a dysfunctional kidney, upregulation of the HIF pathway raises concerns in tumorigenesis via VEGF-mediated angiogenesis. To mitigate the risks in CKD patients who are susceptible to cancer, potential strategies include combining HIF-PHIs with anti-VEGF agents and HIF-dependent PD-L1 blockers. Gene editing techniques like CRISPR/Cas 9 are actively under research to achieve selective and tissue-specific downregulation of particular prolyl hydroxylase subtypes. Due to limited space, this article did not elaborate on hepcidin antagonists, recombinant human EPO and EPO receptor agonists, which are alternative anemia-correcting medications. As a more comprehensive review of management approaches for CKD anemia, future research will look into these drugs and further explore the regulatory pathways of HIFs and their associated genes, with a goal to optimize the safety profile of current HIF-PHIs.

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