Clinical Kidney Journal

Clinical Kidney Journal, 2024, vol. 17, no. 3, sfae051

https://doi.org/10.1093/ckj/sfae051
Advance Access Publication Date: 28 February 2024
CKJ Review

CKJ REVIEW

Growing concerns about using hypoxia-inducible factor prolyl hydroxylase inhibitors for the treatment of renal anemia

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ABSTRACT

Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) have emerged as a novel therapeutic class for treating anemia in patients with chronic kidney disease. Small molecule analogs of α-ketoglutarate (AKG), an essential substrate for 2-oxoglutarate-dependent dioxygenases (2-OGDDs), including prolyl hydroxylase domain proteins (PHDs), inhibit PHDs pharmacologically and thereby prevent HIF degradation. HIF stabilization alleviates anemia through several stimulatory effects on erythropoiesis, but it also affects the expression of many anemia-unrelated genes whose protein products exert important functions *in vivo*. Therefore, the pleiotropic effects of HIF stabilization under normoxic conditions deserve to be examined in more detail. Specifically, we believe that particular attention should be given to epigenetic modifications among the various AKG-based metabolic systems that may be altered by HIF-PHIs. It is noteworthy that AKG has been reported to exert health-protective actions. AKG-based metabolic systems include enzymes associated with the tricarboxylic acid cycle and amino acid metabolism, as well as 2-OGDD-mediated processes, which play important roles in many biological reactions. In this review, we examine the multifaceted effects of HIF-PHIs, encompassing not only their on-target effect of HIF stabilization but also their off-target inhibitory effects on various AKG-based metabolic systems. Furthermore, we examine its potential relevance to cardiovascular complications, based on clinical and animal studies suggesting its involvement in vascular calcification, thrombogenesis and heart failure. In conclusion, although HIF-PHIs offer a promising avenue for anemia treatment in CKD patients, their broader impact on multiple biological systems raises substantial concerns. The intricate interplay between HIF stabilization, AKG competition and cardiovascular complications warrants extensive, long-term investigations to ensure the safety and usefulness of HIF-PHIs in clinical practice.

Keywords: 2-oxoglutarate-dependent dioxygenases, α-ketoglutarate, epigenetics, HIF-PHIs, renal anemia

INTRODUCTION

Anemia is a common complication in chronic kidney disease (CKD) patients. Clinical practice guidelines recommend the use of recombinant human erythropoietin (rhEPO) as an erythropoiesis-stimulating agent (ESA) and iron supplementation for its treatment [1]. Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) have emerged as a new class of

Received: 15.2.2024; Editorial decision: 27.2.2024

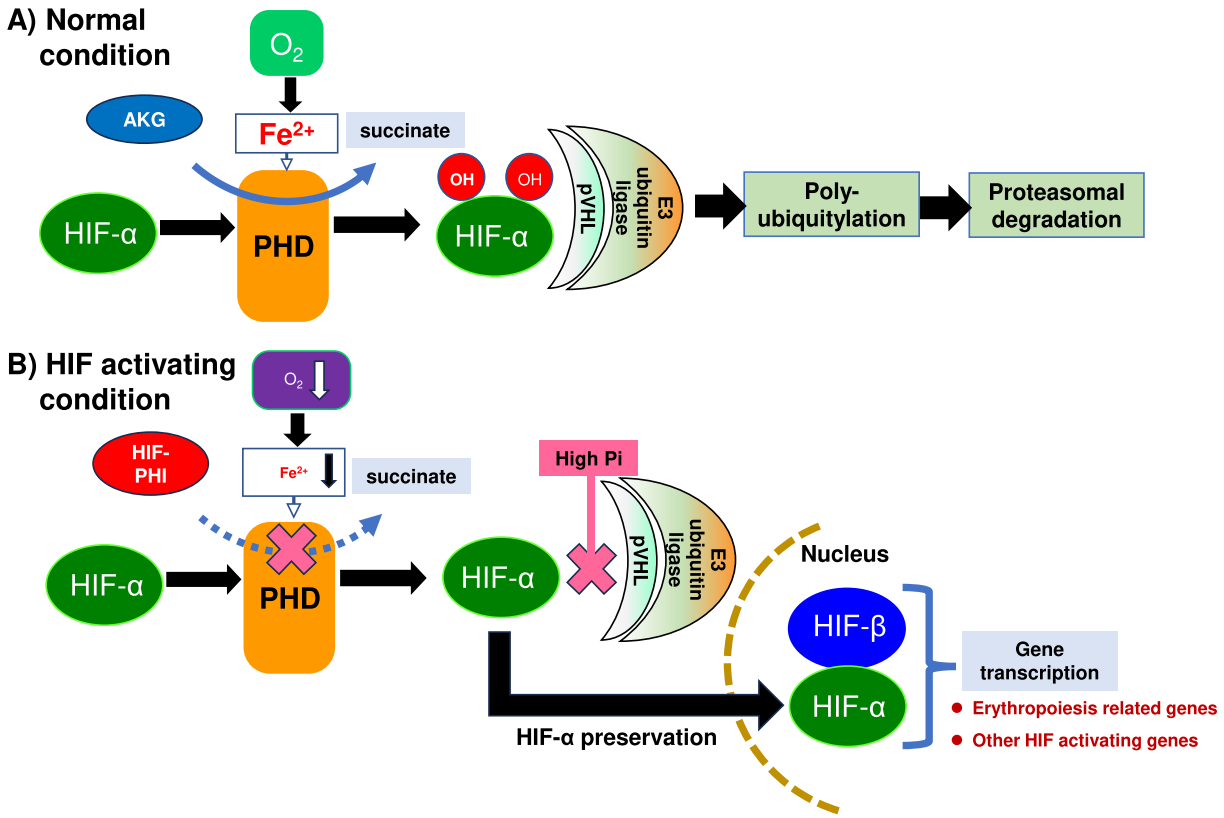

Figure 1: HIF-α metabolism under (**A**) normal and (**B**) HIF-activating conditions. (A) The oxygen-sensitive α-subunit of HIF (HIF-α) is constitutively synthesized. Under normal conditions, specific proline residues within HIF-α are immediately hydroxylated by the PHD, allowing recognition by the von Hippel–Lindau (VHL) ubiquitin–E3 ligase complex and subsequent degradation by the ubiquitin–proteasome pathway. (B) PHD enzymes require dioxygen and 2-oxoglutarate as substrates and ferrous iron at their catalytic centers. Deficiency of any of these factors inhibits PHD activity. Therefore, not only hypoxia but also 2-oxoglutarate or iron deficiency inhibits the degradation of HIF-α, resulting in the retention of HIF-α and an increase in HIF transcription factor in the nucleus. This increases the transcription of HIF-regulated genes involved in a variety of biological processes, including erythropoiesis. HIF-PHIs are analogs of AKG that act as small-molecule competitive antagonists for PHD. In addition, high phosphate concentrations might prevent HIF-α from binding to VHL, resulting in the conservation of HIF-α.

therapeutic molecules. The stimulation of erythropoiesis by HIF-PHIs mainly depends on HIF-α stabilization. Prolyl hydroxylase domain proteins (PHDs) play an essential role in the degradation of persistently produced HIF-α. They exist in three isoforms (PHD1, PHD2 and PHD3), and are dioxygenases that catalyze HIF-α hydroxylation using molecular oxygen and α-ketoglutarate (AKG), also known as 2-oxoglutarate (2-OG), as substrates in an iron-dependent manner [2, 3]. Under normoxic conditions, HIF-α subunits are continuously hydroxylated by PHD enzymes [4]. This reaction initiates their degradation via von Hippel–Lindau-mediated ubiquitylation and blocks the formation of HIF transcription factors [2, 3]. HIF-PHIs are small molecule analogs of AKG, which is an essential substrate for PHD. The pharmacological inhibition of PHD by HIF-PHIs [3] results in a transient "pseudohypoxic" state, stabilizing HIF even under normoxic conditions. Stabilization of HIF improves anemia by affecting both erythropoiesis and iron metabolism (Fig. 1). However, HIF-PHIs may affect many other important metabolic systems in addition to improving anemia.

In this review, we will discuss not only the HIF stabilizing effects of HIF-PHIs (on-target effect) but also the systemic response to their action via inhibition of AKG (off-target effect) and its relevance to cardiovascular (CV) complications of CKD.

ADVANTAGES OF AND POTENTIAL ISSUES WITH HIF-PHIS

All commercially available HIF-PHIs, which have been developed for the treatment of anemia in patients with CKD, have been validated and approved to some extent. However, because numerous genes are regulated by the HIF pathway, there is some concern that many other biological responses may be negatively affected. Since HIF-PHIs are small-molecule competitive antagonists of AKG, an irreplaceable biomolecule with pleiotropic activity at the center of a wide range of physiological processes in addition to cellular metabolism [3, 5], it is possible that AKG antagonists inhibit ubiquitous enzymatic reactions that use AKG as a substrate.

Despite initially promising results in anemia treatment this is probably the reason why several national health authorities have withheld approval, based on unfavorable Phase 3 clinical trial outcomes [3, 6, 7]. Regarding the clinical application of HIF-PHIs for renal anemia treatment, Phase 1 and Phase 2 studies of roxadustat were published in 2015 [8], but these studies have received a withhold recommendation from the US Food and Drug Administration (FDA) [3]. Each country handles market approvals differently; to date, several countries have approved the clinical use of several HIF-PHIs. The US FDA approved

daprodustat as the first oral treatment for anemia caused by CKD in adults who have been receiving dialysis for at least four months. The European Medicines Agency approved the three molecules, roxadustat, vadadustat and daprodustat (vadadustat and daprodustat only in dialysis patients, the latter having been withdrawn by the respective pharmaceutical company for commercial reasons) [9]. In Japan, however, an unprecedented number of five HIF-PHIs including roxadustat, vadadustat and daprodustat have been approved, possibly because the review process differs from that in many other countries. The Japan Pharmaceuticals and Medical Devices Agency (PMDA) did not request CV outcome trials for the submission of PHD inhibitors [10]. According to the PMDA, the rate of CV events is markedly lower in Japan than in Western countries [11], and clinical trials have shown no evidence of a greater CV risk with HIF-PHIs than with rhEPO. Instead, the PMDA is enhancing post-marketing surveillance to uncover potential risks based on real-world evidence. Therefore, it should be noted that some drugs that are not intended for submission to other regions are marketed solely on the basis of their efficacy in treating anemia.

In this section, we will discuss several issues regarding the on-target effects of HIF-PHIs and potential concerns about their off-target effects owing to the multiple physiological functions of AKG.

On-target effect of HIF-PHIs: intermittent HIF stabilization under normoxic conditions

Unique features of anemia treatment with HIF-PHIs

HIF is a heterodimeric transcription factor that is composed of two basic proteins, e.g. HIF-α and HIF-β. The HIF-α/-β dimer, as the HIF transcription factor, binds to hypoxia response elements (HREs) in the gene promoter to transactivate multiple target genes that execute the canonical hypoxia response [12]. In the treatment of renal anemia HIF activates several target genes associated with erythropoiesis and iron metabolism, including EPO, EPO receptors, transferrin, the transferrin receptor and others [3, 12]. (Fig. 1)

A unique feature of anemia treatment with HIF-PHIs is that it can improve anemia to the same extent as can rhEPO preparations, even when blood EPO concentrations are two orders of magnitude lower [13]. It is reported that anemia improvement is observed even in chronic inflammatory conditions with high CRP levels at the same dose as in the non-inflammatory state, although those reports used roxadustat [14, 15]. This may be due to the efficient facilitation of the supply of iron to the erythroblast cells linked with activated genes of iron metabolism [16]. In particular, the hepcidin-lowering effect of HIF-PHIs could promote robust erythropoiesis in erythroblasts [17–20], since hepcidin decreases iron delivery from macrophages and the intestine to erythroblasts. However, it has been reported that hepcidin is not a direct transcriptional target of HIF [21]. The mechanism by which hepcidin is decreased by HIF-PHIs has been attributed primarily to increased production of erythroferron due to increased hematopoiesis [22].

Non-anemia related effects of HIF-PHI

In addition to improving anemia, HIFs are also involved in the regulation of a variety of biological processes under physiological and pathological conditions. The list of these target genes has grown progressively [12, 23]. These include genes related to glucose and energy metabolism, angiogenesis, cell migration, cell–cell and cell–matrix interactions, and numerous other metabolic pathways. Notably, these genes, which are associated with "on-target effects" of HIF but are unrelated to the treatment of anemia, may be the cause of complications associated with the use of HIF-PHIs [12, 24]. These include possible involvement in cancer progression, retinal changes, pulmonary hypertension (PH) and cyst formation in adult polycystic kidney disease. The association of these complications with HIF is complex and not fully understood, but recent reports suggest that the first two are strongly associated with vascular endothelial growth factor, while PH is related to proliferation of endothelial colony-forming cells [25] and cyst formation is related to chloride-dependent fluid secretion into the cyst lumen [26]. Phase 3 studies of HIF-PHIs, which examined carcinogenicity, retinal changes and other potential issues, have demonstrated efficacy and safety at least for a limited time period [6, 7, 27].

HIFs have been implicated in a rapid, reversible metabolic switch from carbohydrate oxidation to anaerobic glycolysis in association with a reduction of mitochondrial performance [28, 29]. The concomitant reduction in oxygen consumption may in turn be associated with decreased mitochondrial generation of cellular damage triggering reactive oxygen species (ROS) [30]. However, in a report using a mouse model of ischemic skeletal muscle, mitochondria continued to consume oxygen as a protective mechanism even in early-stage hypoxic conditions, resulting in excessive ROS generation despite the reduction of mitochondrial function by the concomitant stabilization of HIF which also should have reduced ROS production [31]. There are at least two possible explanations for this apparently contradictory situation. One explanation is that the reduction of mitochondrial metabolism by HIF is not sustained or only partially effective. The other is that HIF-1 reduces the number of mitochondria and inhibits mitochondrial metabolism and respiratory functions by inhibiting PPAR coactivator-1 (PGC-1) family members, and the decreased expression of critically important proteins for electron transport amplifies oxidative stress [30, 32]. Since HIF-PHI-induced HIF stabilization is not sustained, but only intermittent under normal oxygen partial pressure conditions, there may be a risk of intermittent rebounds in ROS production.

Furthermore, short-term versus sustained HIF activation under normoxic conditions may have different effects on the organism in physiological and various pathological states. Short-term, intermittent HIF-α activation has been shown to be protective for several organs in regional ischemia models of the heart and the kidney [33–35]. On the contrary, cardiac-specific PHD inactivation resulting in long-term HIF-α activation was associated with ultrastructural, histological and functional changes in a mouse model of heart failure, reminiscent of ischemic cardiomyopathy over time [36].

In light of these observations, ROS generation can be increased when HIF is permanently activated despite the absence of hypoxia, corresponding to a pseudohypoxic situation. In line with this assumption, several reports have pointed to the possible onset and exacerbation of CV disease (CVD) with long-term administration of HIF-PHIs [7]. Whether the long-term HIF-1α stabilization by HIF-PHIs has any negative impact on life expectancy needs to be investigated by long-term studies.

Off-target effects of HIF-PHIs: presumed competition with AKG, a metabolite with multiple functions

As mentioned above, AKG is central to a variety of metabolic systems [37]. It plays a key role as a tricarboxylic acid (TCA)

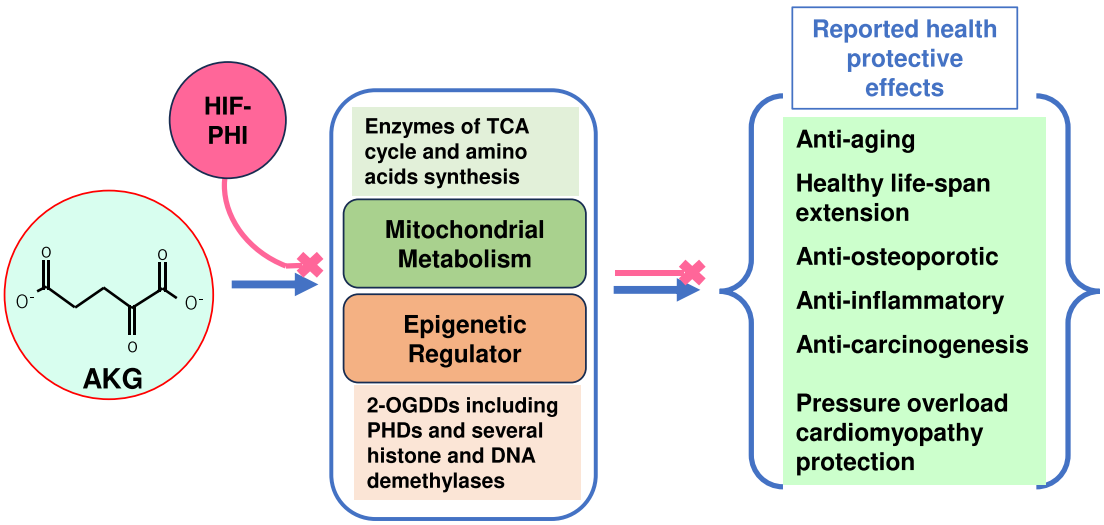

Figure 2: Health-protective effects of AKG. There is increasing evidence for multiple health-protective effects of AKG. AKG is an important intermediate in the TCA cycle and is involved in the biosynthesis of amino acids in mitochondria. AKG is also an obligatory co-substrate for 2-OGDDs. The 2-OGDDs are a diverse group of enzymes that include PHD proteins and several histone and DNA demethylases, which are epigenetic regulators. The multiple health protective effects of AKG could be related to mitochondrial metabolism as well as epigenetic regulators. Given that HIF-PHIs are small-molecule analogs of AKG, they may suppress the health-protective effects of AKG.

cycle metabolite linked to ATP production and reducing equivalent (NAD^+/NADH) generation, which in turn can influence ROS generation [38]. AKG is also a precursor of glutamine and regulates amino acid synthesis [5, 37, 38].

Even more noteworthy is that AKG is also an obligatory co-substrate for 2-oxoglutarate-dependent dioxygenases (2-OGDDs) [39, 40]. 2-OGDDs are a diverse group of enzymes that facilitate the oxidation of C-H bonds in various organic compounds and catalyze hydroxylation reactions on various types of substrates, including proteins, nucleic acids, lipids and metabolic intermediates associated with multiple cellular metabolic and regulatory pathways [39, 40]. It is estimated that humans have 60–70 2-OGDDs [41], including HIF-PHDs and histone and DNA demethylases, i.e. Jumonji C domain-containing histone demethylases (JMJDs), and 10–11 translocation hydroxylases (TET1–3). 2-OGDDs are receiving increased attention due to their expanding role in genetic regulation and stress response signaling [42, 43]. They are involved in multiple steps of gene expression, from transcription factor regulation and epigenetic control of chromatin structure to RNA stability and protein translation [38].

Regarding HIF-PHDs, HIF-PHIs occupy the AKG binding site and inhibit PHDs, thereby inhibiting O_2-dependent degradation of HIF-1α and HIF-2α, as mentioned above [3, 44]. Similarly, HIF-PHIs may inhibit other 2-OGDDs. Given the pleiotropic and complex nature of the many biological reactions involving multiple 2-OGDDs, clinically used inhibitors of PHDs must be as selective as possible for the desired physiological outcome, especially in the long-term treatment of chronic diseases such as the anemia of CKD patients. Pharmaceutical companies have, of course, investigated the specificity of HIF-PHIs but have not disclosed them publicly [45]. To our knowledge, the only published study that examined the specificity of clinically used HIF-PHIs for several 2-OGDDs seems to indicate that this approach is not highly specific for PHD [46]. If it is not possible to achieve sufficient selectivity with respect to erythropoietin response, there may be adverse biological effects other than HIF stabilization that could be considered “off-target effects” [47].

The biological off-target effects of HIF-PHIs are theoretically immense, with potentially serious consequences of their long-term use in clinical practice. Of particular concern is, in our opinion, the unknown impact of HIF-PHIs on protein expression via epigenetics, which is not yet fully understood or elucidated. The impact of their long-term use is not necessarily revealed after short-term administration.

In recent years, several health-promoting AKG effects have been reported, including anti-aging [48–50], healthy life-span extension [48], anti-osteoporotic [51, 52], anti-inflammatory [48, 53] and anticarcinogenic effects [5], as well as protection against pressure overload cardiomyopathy [54, 55] (Fig. 2). These health-promoting effects are not only metabolic but also epigenetically mediated [5, 37, 51, 53]. Therefore, there is a concern that HIF-PHIs may interfere with these protective effects on health. Longer-term observations will be needed to determine the impacts of HIF-PHIs on these effects, so future research is warranted. Similar concerns have been raised in several reports, and it is believed that a Phase 3 study of limited duration is not sufficient to ensure long-term safety [46, 56].

SUSPECTED CV ABNORMALITIES LINKED TO HIF-PHIS

Based on these concerns we will discuss the CV complications which may occur based on the observations made in available Phase 3 trials and animal studies, namely, vascular calcification, thromboembolism and heart failure.

Vascular calcification

Vascular calcification is a common complication of CKD. Its extent and histopathologic type are predictors of CV mortality [57] because they cause a loss of arterial elasticity and hence increase arterial stiffness, resulting in the development of left ventricular hypertrophy, decreased coronary artery perfusion, myocardial ischemia and heart failure [58, 59].

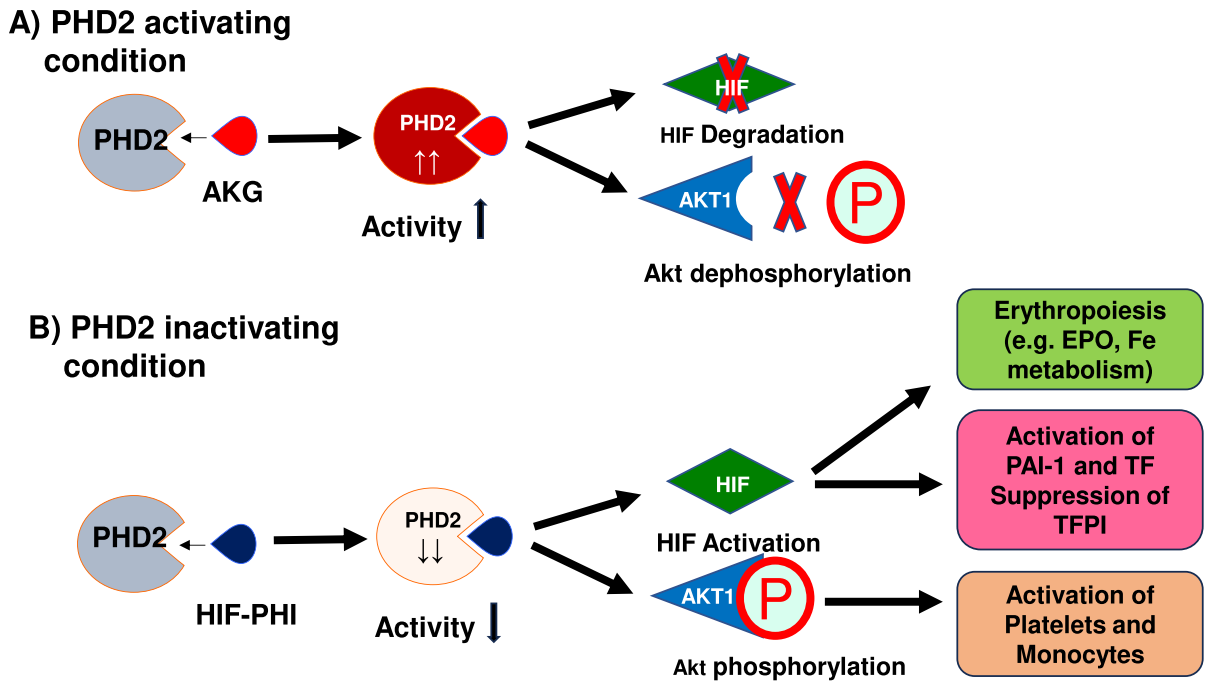

Figure 3: Thromboembolic events associated with HIF-PHIs. (**A**) PHD protein activation conditions. PHD2 is activated by AKG. For HIF-PHD, PHD2 hydroxylates constitutively synthesized HIF-α and leads to degradation. Phosphorylation of an isoform of Akt1 has been reported to contribute to platelet and monocyte activation and is implicated in the pathogenesis of thrombosis. Activation of PHD2 also causes Akt1 dephosphorylation. (**B**) Inactivation of the PHD protein. HIF-PHIs suppress PHD2, resulting in HIF stabilization and Akt1 phosphorylation. Certainly, HIF stabilization activates EPO synthesis and improves iron metabolism, which causes erythropoiesis. It also activates TF and PAI-1 and suppresses the antithrombotic TFPI, which could cause thrombosis. Inhibition of PHD2 activity prevents the phosphorylation of Akt1, which promotes thrombus formation by activating platelets and monocytes. The circled letter P indicates phosphorylation.

A previous *in vitro* study using vascular smooth muscle cells highlighted the potential role of hypoxia and HIF-1 pathway activation in vascular calcification [60]. HIF-1α may accelerate calcium deposition by increasing the expression of the master regulator of osteogenic differentiation, RUNX2. Elevated levels of inorganic phosphate (Pi) induce the conservation of HIF-1α by preventing the interaction between hydroxylated HIF-1α and PHD proteins and the von Hippel–Lindau protein for polyubiquitylation [60]. Thus, HIF-1α degradation is suppressed by high Pi conditions, leading to increased HIF-1α stabilization even in the absence of hypoxia [60, 61].

Recent *in vivo* studies have also demonstrated a vascular calcification–promoting effect of HIF-PHIs. In mice with CKD on an adenine and high-phosphate diet daprodustat increased aortic calcification assessed using osteosense dye, a technique which allows revealing areas of osteogenesis that are not detected by von Kossa staining [62].

Patients with kidney failure often have advanced vascular calcification, and it may be difficult to demonstrate an increase in calcium deposition caused by HIF-PHI administration in them. So far there is no report on a possible enhancement of vascular calcification by such treatment in human patients. However, given that HIF-PHIs have been shown to promote vascular calcification in mouse models, the possibility that this may also occur in patients with kidney failure should be strongly considered.

Thromboembolism

According to the network meta-analysis of 26 randomized controlled trials with 14 945 patients, in general the thrombogenic risk of HIF-PHI treatment is greater than that of conventional ESAs, although there are differences among HIF-PHI subtypes [19]. Specifically, roxadustat caused more thrombosis than ESAs and vadadustat according to this analysis, and the lowest rates of thrombosis were seen with molidustat and ESAs. The US FDA has also raised concerns surrounding thrombotic risk of certain HIF-PHIs [7].

The causes of thrombosis are complex. They involve not only elevated coagulation factor levels but also platelet activation and several other yet unknown factors [63]. The procoagulant factors include protein products of genes for which there is evidence of direct transcriptional activation by HIF, which directly regulate coagulation and fibrinolysis, such as prothrombotic tissue factor (TF, factor III), plasminogen activator inhibitor-1 (PAI-1) and antithrombotic TF pathway inhibitor (TFPI) [64–67] (Fig. 3). TF regulates the extrinsic coagulation system. Dysregulation of TF is associated with abnormal hemostasis and an increased risk of thrombotic events [65, 68]. PAI-1 is an important suppressor of fibrinolysis involved in coagulation control [66, 69]. TFPI is a serine protease inhibitor that regulates the tissue factor-dependent blood coagulation pathway. HIF-2 suppresses TFPI and thereby enhances the pro-thrombotic potential in endothelial cells [65, 70].

Another recent report suggested that not only these factors but also PHD itself, the target of HIF-PHIs, may be involved in the suppression of thrombus formation via hydroxylation of serine-threonine kinase Akt [71]. This kinase, also known as protein kinase B (PKB), has been reported to contribute to platelet and monocyte activation. Among the three identified isoforms, Akt1 is widely expressed in most human and mouse cells [72, 73]. Phosphorylation of Akt1 (pAkt1) by membrane-associated 3-phosphoinositide-dependent kinase-1 (PDK1)

plays an important role in platelet functional responses, including aggregation, adhesion and thrombus formation [71, 74] (Fig. 3). PHD2 can hydroxylate pAkt1, leading to dephosphorylation and inactivation of the kinase. When the effects of AKG (a cofactor of PHD2) and dimethyl ketoglutarate (DKG, an inhibitor of PHD2) on platelet aggregation and pAKT1 levels were tested in a collagen adhesion platelet thrombus formation assay using whole blood from healthy control individuals it was shown that AKG attenuated platelet aggregation by inactivating pAkt1, whereas DKG enhanced platelet aggregation by promoting its phosphorylation [71, 75]. Similarly, in mice treated with the thrombogenic agent carrageenan AKG reduced clot formation and leukocyte accumulation in several organs, including the lung [71].

Severe forms of the coronavirus disease 2019 (COVID-19) pandemic may present with the feature of coagulopathy as a component of systemic inflammatory response syndrome. In severely infected individuals pulmonary thrombotic microangiopathy may lead to lung failure [76, 77]. Such fatal outcome could be successfully prevented in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected hamsters by dietary supplementation with the PHD2 activator AKG, rescuing the inflamed lungs by significantly reducing leukocyte accumulation, clot formation and viral load via downregulation of pAkt [78]. Consequently, PHD enzyme inhibition, in contrast to PHD activation, may increase the potential of thrombus formation. To our knowledge, there are no reports on the prognosis of CKD patients with COVID-19 who were receiving HIF-PHI therapy. However, preliminary results of a randomized controlled trial suggest that treatment with the HIF-PHI desidustat could potentially help to prevent acute respiratory distress syndrome in patients with COVID-19, although there was no information on kidney function [79].

Several previous reports have suggested that HIF-PHI treatment may be associated with iron deficiency because of less iron administration and that this in turn could induce thrombus formation via enhanced transferrin production [7]. The stabilization of HIF via decreased PHD activity associated with iron deficiency is currently the most plausible mechanism for increased transferrin production [80]. Therefore, it is not unreasonable to assume that iron deficiency enhances HIF stabilization in patients treated with HIF-PHIs, leading to further transferrin production. Mice kept in hypoxic conditions similar to those experienced by humans living at high altitude developed elevated transferrin and coronary thrombi. The impact of hypoxia on thrombosis was attenuated using transferrin-specific antibodies, inhibitory peptides and transferrin knockdown [81]. However, this is in apparent contradiction with a recent observation in patients with Chuvash erythrocytosis in whom transferrin elevation were unexpectedly associated with reduced rather than increased thrombosis risk [82]. At present, it remains unclear whether high-dose iron supplementation contributes or not to thrombogenesis in patients with CKD receiving HIF-PHI treatment.

Heart failure

The US FDA has approved daprodustat for the treatment of anemia exclusively in adults with CKD who have been receiving dialysis for at least 4 months. When reviewing the effects of daprodustat in patients with CKD not yet on dialysis therapy in the Anemia Studies in Chronic Kidney Disease: Erythropoiesis Via a Novel Prolyl Hydroxylase Inhibitor Daprodustat-Non Dialysis (ASCEND-ND) trial the participants at the Cardiovascular and Renal Drugs Advisory Committee Meeting noted an increase in the estimated hazard ratio (HR) for hospitalization for heart failure in patients with a history of heart failure, even though patients with a history of severe heart failure [New York Heart Association (NYHA) IV] were excluded from the trial [83] (https://www.fda.gov/media/162607/download). It is also worth noting that the same type of analysis done in the dialysis patient population of the corresponding Anemia Studies in Chronic Kidney Disease: Erythropoiesis Via a Novel Prolyl Hydroxylase Inhibitor Daprodustat-Dialysis (ASCEND-D) trial was barely statistically significant [84].

With the use of HIF-PHIs in patients with heart failure, we speculate that disturbed cardiac energy metabolism may be a cause of increased risk of cardiac decompensation [85, 86]. The normal myocardium produces most (95%) of its ATP requirements through oxidative phosphorylation within the mitochondrion-anchored electron transport chain (ETC) [87–89]. Fatty acids are the major energy-producing substrates, but other substrates such as glucose and ketones are also used for energy production. Acetyl-CoA is produced by fatty acid and glucose oxidation and enters the mitochondrial TCA cycle. This leads to the synthesis of NADH and $FADH_2$, which are used by the ETC to synthesize ATP.

In the state of heart failure, cardiac energy production is markedly reduced, with the primary energy substrate being switched from fatty acids to glucose, although a large amount of acetyl-CoA is produced from fatty acids through repeated cycles of β-oxidation [90, 91]. In addition, since oxygen supply is severely reduced in advanced heart failure a shift from aerobic to anaerobic metabolism occurs. This in turn shifts ATP production from the ETC to the glycolytic system together with HIF activation [30, 89]. In the ETC system, mitochondria produce more than 30 molecules of ATP from one molecule of glucose, whereas the glycolytic system in the cytoplasm can produce only two molecules of ATP from the same amount of glucose through oxygen-independent enzymatic reactions [89, 90] (Fig. 4). These changes limit mitochondrial metabolism, resulting in a state of greatly reduced energy production instead of reduced oxygen consumption, which can reduce oxidative stress [91]. Recent *in vivo* studies in a rat model of aortic stenosis and chronic heart failure have suggested that HIF-1α is partly responsible for these metabolic alterations, although other underlying mechanisms may play a role as well [92]. Based on these considerations we hypothesize that the administration of HIF-PHIs to patients with heart failure is likely to further exacerbate the imbalance between ATP demand and supply.

In line with this hypothesis it has been recently shown that AKG exerted cardiomyocyte protective effects in a mouse model of heart failure induced by transverse aortic constriction [54]. AKG supplementation to these animals ameliorated cardiac morphological features, including organ weight, cell size and fibrosis by increasing mitophagy allowing to clear damaged mitochondria and to attenuate myocardial oxidative stress under pressure overload. Using same model, another group of authors showed that ablation of oxoglutarate receptor 1 (OXGR1, receptor for AKG) in cardiomyocytes resulted in impaired cardiac function and hypertrophy, whereas OXGR1 overexpression reduced phenylephrine-induced hypertrophy [93]. Therefore, the potential inhibition of AKG-associated metabolic systems by HIF-PHIs may be of concern in heart failure.

Since the incidence of heart failure in CKD patients is high we suggest that HIF-PHIs should be used with greater caution [94]. In particular, we would posit that it may be safe to withhold HIF-PHIs from patients with severe heart failure (NYHA IV) unless long-term experience with this new class of agents convincingly demonstrates that such caution is not warranted.

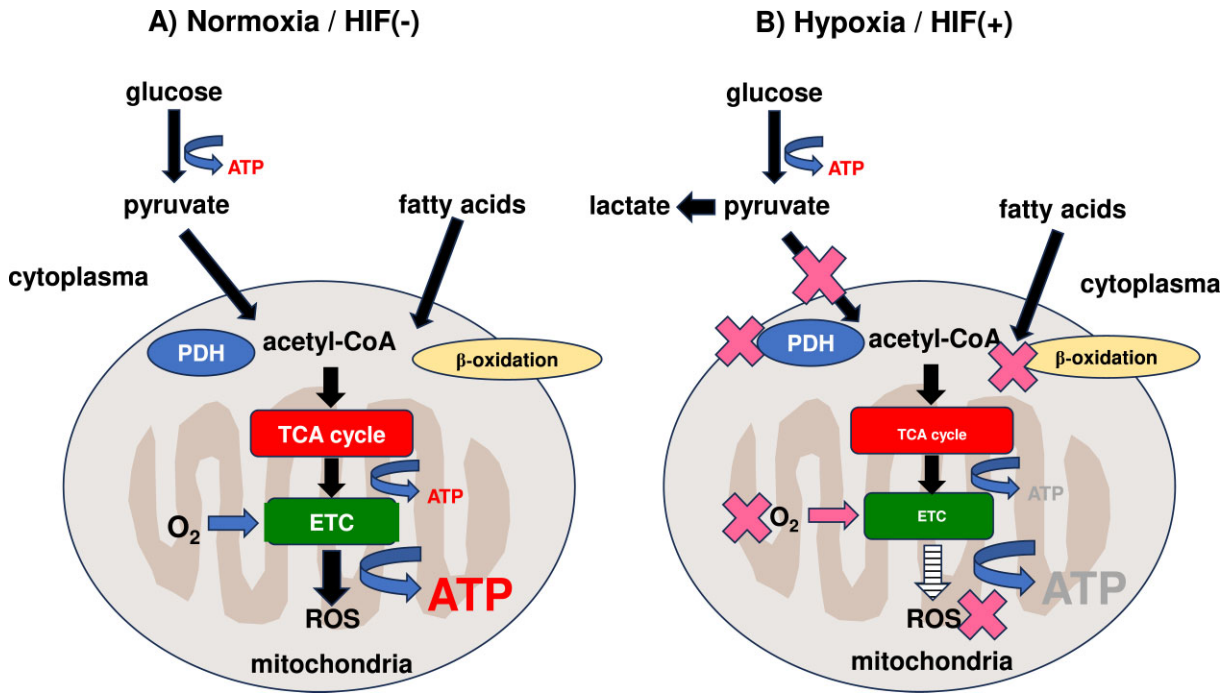

Figure 4: ATP synthesis under conditions of HIF stabilization in the myocardium. (**A**) Under normoxic conditions without HIF stabilization. ATP is essential for cell viability and myocardial pump function. In the heart, fatty acids are the preferred carbon source for the generation of ATP. Acetyl-CoA is generated by fatty acid oxidation and enters the mitochondrial TCA cycle. This leads to the synthesis of NADH and $FADH_2$, which are used by the mitochondrial ETC to synthesize ATP. From fatty acids, an abundance of acetyl-CoA is generated through repeated cycles of β-oxidation. (**B**) Under hypoxic conditions with HIF stabilization. Fatty acid oxidation is suppressed, and glycolysis is stimulated by activating glucose transporters and glycolytic enzymes. However, the entry of pyruvate, the end product of glycolysis, into the mitochondria and its conversion to acetyl-CoA by pyruvate dehydrogenase (PDH) are inhibited by HIF-1, resulting in an increase in lactate synthesis. Thus, ATP synthesis should be dependent on glycolysis. Although the generation of ROS may be reduced, any increase in glucose uptake and utilization is not sufficient to compensate for the overall reduction in ATP supply capacity. Reduced energy availability through all these pathways may contribute to impaired contractile reserve under these conditions.

CONCLUSION

HIF-PHIs are small-molecule analogs of AKG which is an indispensable substrate of 2-OGDDs, including PHD. They induce pharmacological inhibition of PHD and thereby in stabilize HIF. HIF stabilization has the advantage of alleviating anemia but it also affects the expression of numerous genes unrelated to erythropoiesis that have important functions in the organism. HIF-PHIs have the potential to alter a large number of AKG-based metabolic systems, such as enzymes associated with the TCA cycle, amino acid synthesis and 2-OGDD-mediated functions, which play important roles in many biological processes. Specific consideration should be given to epigenetic actions. AKG itself has been reported to have health-protective effects including anti-aging effects and lifespan extension. Although the exact underlying mechanisms are still unclear, the possibility that HIF-PHIs interfere in these processes cannot be ruled out. In this review, we attempted to show the multifaceted actions of HIF-PHIs, with special attention to possible off-target effects through AKG inhibition and potential relevance to CV complications. Our concerns are based on clinical and animal studies highlighting the potential contribution of HIF-PHIs to vascular calcification, thrombogenesis and heart failure. Despite great initial promise, national health authorities of several countries have withheld approval, based on partially unfavorable Phase 3 trial results. These are the results that have been shown under the strict exclusion criteria for patients in the majority of the Phase 3 trials. For example, in the ASCEND-ND and ASCEND-D trials, subjects who had recent myocardial infarctions, acute coronary syndromes, stroke, transient ischemic attacks, NYHA functional class IV heart failure, or uncontrolled hypertension were excluded [83, 84, 95]. The intricate interplay between HIF activation, AKG competition and CV complications warrants the performance of extensive, long-term studies to ensure safety and efficacy of HIF-PHI treatment in clinical practice. As roxadustat is undergoing post-marketing surveillance in Japan (NCT04408820) to track long-term outcomes, publication of these results is awaited.

ACKNOWLEDGEMENTS

We gratefully acknowledge the advice and editorial assistance of Tilman B. Drueke, MD, Inserm Unit 1018, CESP, Villjuif/Paris, France.

DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

CONFLICT OF INTEREST STATEMENT

T.N. reports personal fees from Kyowa-Kirin and Kissei. T.K. reports personal fees from Chugai, personal fees from

Kyowa-Kirin, personal fees from Astellas, personal fees from Bayer Yakuhin, grants and personal fees from Ono, personal fees from Fuso, grants from Kissei and personal fees from Tanabe Mitsubishi, personal fees from Baxter Ltd, personal fees from AstraZeneca KK, personal fees from Torii Pharmaceutical Co., Ltd, personal fees from Sanwa Kagaku Kenkyusho Co., Ltd, and personal fees from Kaneka Medical products, outside the submitted work.

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Received: 15.2.2024; Editorial decision: 27.2.2024