The role of hypoxia-inducible factor stabilizers in the treatment of anemia in patients with chronic kidney disease

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Introduction: The purpose of this study was to analyze the effects of hypoxia-inducible factor (HIF) stabilizers on anemia in non-dialysis-dependent (NDD) and dialysis-dependent (DD) chronic kidney disease (CKD) patients.

Methods: Published studies were extracted from PubMed, China Biological Medicine Database (CBM), Wanfang database, and Cochrane Library on March 10, 2018, and relevant studies were pooled and included in a meta-analysis. Data on hemoglobin (Hb), ferritin, and hepcidin levels, total iron-binding capacity (TIBC), and incidence of adverse events (AEs) were extracted and pooled using Review Manager Version 5.3.

Results: Data from nine selected studies were extracted. Meta-analysis of the included studies showed that HIF stabilizers reduced ferritin and hepcidin levels and increased Hb level and TIBC in NDD-CKD patients. However, HIF stabilizers only increased TIBC, and did not affect ferritin, hepcidin, and Hb levels in DD-CKD patients. Furthermore, no notable differences in AEs and severe AEs between NDD-CKD and DD-CKD patients were detected.

Conclusion: HIF stabilizers are effective for the treatment of anemia in NDD-CKD patients and safe for short-term use.

Keywords: hypoxia-inducible factor stabilizer, anemia, chronic kidney disease, meta-analysis

Introduction

Chronic kidney disease (CKD) has become a global public health problem and results in significant morbidity and mortality.1,2 Anemia is one of the hallmarks of advanced CKD and is correlated with a lower quality of life and increased mortality.3-5 Renal anemia develops secondary to CKD, and its incidence increases with CKD progression. With the development of disease, patients inevitably develop end-stage renal disease, which often requires dialysis or kidney transplantation.6 Anemia is a common complication of CKD.7 Currently, recombinant human erythropoietin (rhEPO) is a cornerstone in the treatment of anemia associated with CKD. In the past, super-physiological doses of erythropoiesis-stimulating agents were employed to treat anemia; however, this has been associated with risk of cardiovascular events, as well as induces endothelial dysfunction.8

Hypoxia-inducible factor (HIF) is a heterodimer composed of HIF-α and HIF-β subunits.9 HIF-β is constitutively expressed, whereas HIF-α is modulated by oxygen tension via a family of HIF-prolyl hydroxylases (PHDs) regulating its degradation by the proteasome.10 During hypoxia, HIF plays an important role in survival by regulating the levels of erythropoietin (EPO), glucose transporter-1, vascular endothelial
growth factor, and pyruvate dehydrogenase kinases 1 and 4,11
HIF-prolyl hydroxylase inhibitors (PHIs) are small-molecule
oral agents that stabilize HIF, resulting in the activation of
HIF-mediated gene expression.12 With increased HIF levels,
concomitant expression of downstream molecules, such as
EPO, occurs. HIF-1α induces the production of inflammatory
cytokines and autoantibodies by promoting the persistence of
interactions between synovial fibroblasts, T cells, and B cells.
Therefore, targeting HIF-1α may be a potential therapeutic
strategy for persistent disease.13 Anemia secondary to CKD
is a type of chronic inflammatory anemia,14,15 and HIF
stabilizers may improve the inflammatory status of CKD
patients.16 Despite this theory, the therapeutic use of HIF
stabilizers in the clinic has not been thoroughly analyzed.
This meta-analysis was performed to assess the effects of
HIF stabilizers on anemia resulting from CKD.

Materials and methods

Search strategy
The website www.ClinicalTrials.gov was queried to deter-
mine the HIF stabilizers that have been investigated in
clinical trials up to March 10, 2018. The keywords roxadu-
stat, vadadustat, daprodustat, and molidustat were entered
into PubMed, China Biological Medicine Database (CBM),
Wanfang database, and Cochrane Library, and relevant
studies were identified without any language limitation. The
references cited in the recruited articles were also checked
to identify additional reports.

Inclusion and exclusion criteria

Inclusion criteria
The inclusion criteria for the studies were as follows: 1) study
type: randomized controlled trials, randomized crossover
studies, and prospective studies; 2) study subjects: patients
who met the diagnostic criteria only for anemia of CKD,
regardless of race; 3) interventions: HIF stabilizer (roxadu-
stat, vadadustat, daprodustat, molidustat) for treatment; and
4) outcome: different HIF stabilizers were compared with
placebo or traditional drugs.

Exclusion criteria
Exclusion criteria for the studies were as follows: 1) the study
focused on primary anemia or anemia secondary to other
causes, such as blood loss, blood diseases, and infectious
diseases; 2) the clinical trial involved healthy individuals;
3) the study only described individuals treated with HIF
stabilizers and did not include a reference group; and 4) the
diagnostic criteria were not clear.

Outcome measures
Hemoglobin (Hb; g/L or g/dL), ferritin (ng/mL or µg/L), and
hepcidin (ng/mL or µg/L) levels, total iron-binding capacity
(TIBC; µg/dL or µmol/L), and adverse events (AEs) were
used as outcome measures.

Data collection
According to the predetermined inclusion criteria, two
observers scanned the titles and abstracts, or read the full
text to screen out possible relevant literature. Discordant opinions
were resolved by the other reviewers. Only randomized con-
trolled trials, randomized crossover studies, and prospective
studies that were related to HIF stabilizer treatment were
included in the analysis.

Statistical analysis
Using Review Manager Version 5.3 software, data were
extracted from the included literature. On the basis of the
heterogeneity test results, a fixed-effects model was used
when the P-value was ≈0.1; otherwise, a random-effects
model was used. The results were expressed as weighted mean
differences for continuous data, and additionally, 95% CIs
were calculated. Heterogeneity between included studies was
assessed using chi-squared test with an alpha of 0.05 indicating
statistical significance. Subgroup analysis was conducted to
explore the underlying causes of heterogeneity in treatment out-
comes. The following factors were identified for analysis in the
experimental (HIF stabilizers) and control groups: 1) a change
in mean Hb levels from baseline (ΔHb), 2) a change in mean
ferritin levels from baseline (Δferritin), 3) a change in mean
hepcidin levels from baseline (Δhepcidin), and 4) AEs between
the experimental group and control group. For all analyses,
a two-tailed P-value <0.05 indicated statistical significance.

Results

Search results
In this meta-analysis, nine articles,17–25 describing 12 clinical
trials, related to use of HIF stabilizers in anemia due to
CKD (Table 1) were included. There were five multicenter,
randomized, blinded, placebo-controlled studies17–19,21,22
and three randomized, double-blinded, placebo-controlled
studies.20,23,24 Moreover, the meta-analysis included two open-
label studies.20,24 These nine studies comprised 353 cases and
234 controls (Table 1).

ΔHb values between the HIF stabilizer
and placebo groups
All the nine reports17–25 involved comparison of ΔHb levels
between the case and placebo groups. ΔHb was statistically
significant between the experimental and control groups ($P<0.00001$; Table 2). The $P$-value of the heterogeneity test was $<0.00001$, so a random-effects model was chosen. The pooled mean difference was 2.70 (95% CI: 1.79–3.61). A subgroup analysis was also conducted between NDD-CKD patients and DD-CKD patients to analyze the causes of heterogeneity. For the NDD-CKD patients, seven reports that compared the ΔHb levels between the HIF stabilizer and placebo groups were included. The $P$-value of the heterogeneity test was $<0.00001$, so a random-effects model was chosen. The pooled mean difference was 1.20 (95% CI: 0.65 to 2.51). The difference in the ΔHb levels between the HIF stabilizer and control groups among the DD-CKD patients was not statistically significant ($P=0.07$; Table 2 and Figure 1B).

**ΔFerritin values between the HIF stabilizer and placebo groups**

Nine reports including data from 11 trials were included in this meta-analysis for the assessment of Δferritin levels. The pooled mean difference between the case and control groups was $-0.65$ (95% CI: $-1.12$ to $-0.18$). The difference in the Δferritin levels between the experimental and control groups was statistically significant ($P=0.006$; Table 2). The $P$-value of the heterogeneity test was $<0.00001$, prompting
us to utilize a random-effects model, and an additional subgroup analysis was conducted.

For the NDD-CKD patients, six reports were included in the meta-analysis for comparing the Δferritin levels between the HIF stabilizer and placebo groups. The P-value of the heterogeneity test was <0.00001, so a random-effects model was chosen. The pooled mean difference was -1.12 (95% CI: -1.92 to -0.32). The difference in Δferritin levels between the HIF stabilizer and placebo groups among the NDD-CKD patients was statistically significant (P=0.006; Table 2 and Figure 2A). This indicates that Δferritin levels in the HIF stabilizer group

### Table 2  Meta-analysis of the association of HIF stabilizers with anemia due to chronic kidney disease

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Group and subgroups</th>
<th>Number of studies</th>
<th>Q test P-value</th>
<th>Model selected</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
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<tr>
<td>Hb</td>
<td>Overall</td>
<td>12</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>2.70 (1.79–3.61)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td></td>
<td>NDD-CKD</td>
<td>7</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>3.51 (2.20–4.82)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td></td>
<td>DD-CKD</td>
<td>5</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>1.20 (0.12 to 2.51)</td>
<td>0.07</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Overall</td>
<td>11</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>0.05</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>NDD-CKD</td>
<td>6</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>1.07</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>DD-CKD</td>
<td>5</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>1.03</td>
<td>0.05</td>
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<tr>
<td>Hepcidin</td>
<td>Overall</td>
<td>8</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>1.07</td>
<td>0.01</td>
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<tr>
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<td>NDD-CKD</td>
<td>5</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>1.07</td>
<td>0.01</td>
</tr>
<tr>
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<td>DD-CKD</td>
<td>5</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>1.07</td>
<td>0.01</td>
</tr>
<tr>
<td>TIBC</td>
<td>Overall</td>
<td>11</td>
<td>&lt;0.00001</td>
<td>Random</td>
<td>1.64 (0.98–2.31)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td></td>
<td>NDD-CKD</td>
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<td>&lt;0.00001</td>
<td>Random</td>
<td>2.05 (1.00–3.10)</td>
<td>0.0001</td>
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<tr>
<td></td>
<td>DD-CKD</td>
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<td>&lt;0.00001</td>
<td>Random</td>
<td>1.30 (0.35–2.24)</td>
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<td>Reverse effect</td>
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<td>SAE</td>
<td>Overall</td>
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<td>0.72</td>
<td>Fixed</td>
<td>1.56 (0.91–2.66)</td>
<td>0.11</td>
</tr>
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</table>

**Abbreviations:** HIF, hypoxia-inducible factor; Hb, hemoglobin; TIBC, total iron-binding capacity; SAE, severe adverse event; NDD-CKD, non-dialysis-dependent chronic kidney disease; DD-CKD, dialysis-dependent chronic kidney disease.

Figure 1: Association between HIF stabilizers and Hb in patients with CKD.

Notes: (A) NDD-CKD subgroup. (B) DD-CKD subgroup.

Abbreviations: HIF, hypoxia-inducible factor; Hb, hemoglobin; CKD, chronic kidney disease; NDD-CKD, non-dialysis-dependent chronic kidney disease; DD-CKD, dialysis-dependent chronic kidney disease.
were lower than the placebo group among the NDD-CKD patients.

For the DD-CKD patients, five reports\cite{17,18,20,21,24} were included in the meta-analysis for assessing the Δferritin levels. The $P$-value of the heterogeneity test was 0.05, so a random-effects model was chosen. The pooled mean difference was $-0.22$ (95% CI: $-0.65$ to $0.21$). The difference in Δferritin levels between the HIF stabilizer and the control group among the DD-CKD patients was not statistically significant ($P=0.32$; Figure 2B and Table 2).

**ΔHepcidin values between the HIF stabilizer and placebo groups**

Six reports\cite{18-20,22-24} including eight clinical trials were included in this meta-analysis for assessing the Δhepcidin levels between the case and placebo groups. The difference in Δhepcidin between the experimental and the control group was statistically significant ($P=0.007$; Table 2). The $P$-value of the heterogeneity test was $<0.00001$, so a random-effects model was chosen. The pooled mean difference was $-2.55$ (95% CI: $-4.60$ to $-0.49$). The difference in Δhepcidin levels between the HIF stabilizer and the placebo group among the NDD-CKD patients was statistically significant ($P=0.02$; Figure 3A and Table 2). This indicates that the Δhepcidin levels in the HIF stabilizer group were lower than the placebo group among the NDD-CKD patients.

For the DD-CKD patients, three reports\cite{19,20,24} were included in the meta-analysis for assessing the Δhepcidin levels. The $P$-value of the heterogeneity test was $<0.1$, so a random-effects model was chosen. The pooled mean difference was $-0.19$ (95% CI: $-0.64$ to $0.26$). The difference in Δhepcidin levels was not statistically significant between the two groups among the DD-CKD patients ($P=0.41$; Figure 3B and Table 2).

**ΔTIBC between the HIF stabilizer and the placebo group**

Nine reports\cite{17-25} including 11 trials were included in this meta-analysis for assessing the ΔTIBC between the case and placebo groups. The difference in ATIBC between the experimental and the control group was statistically significant ($P=0.00001$; Table 2). The $P$-value of the heterogeneity test was $<0.00001$, so a random-effects model was chosen. The pooled mean difference was $1.64$ (95% CI: $0.98$–$2.31$).
In addition, a subgroup analysis comparing the NDD-CKD and DD-CKD patients was performed. For the NDD-CKD patients, six reports\textsuperscript{18,20–23,25} describing the $\Delta$TIBC levels between the HIF stabilizer and placebo groups were included. The $P$-value of the heterogeneity test was <0.0001, so a random-effects model was chosen. The pooled mean difference was 2.05 (95% CI: 1.00--3.10). The difference in $\Delta$TIBC levels between the HIF stabilizer and placebo groups among the NDD-CKD patients was statistically significant ($P=0.0001$; Figure 4A and Table 2). This indicates that $\Delta$TIBC levels in the HIF stabilizer group were higher than the placebo group among the NDD-CKD patients.
For the DD-CKD patients, five reports\textsuperscript{17,18,20,21,24} were included in this meta-analysis for assessing the $\Delta$TIBC levels between the HIF stabilizer and control groups. The $P$-value of the heterogeneity test was $<0.00001$, so a random-effects model was chosen. The pooled mean difference was 1.30 (95% CI: 0.35–2.24). The difference in $\Delta$TIBC levels between the two groups was not statistically significant ($P=0.007$; Table 2 and Figure 4B). This indicates that the differences in $\Delta$TIBC levels between the HIF stabilizer and control groups among the DD-CKD patients were not statistically significant.

## AEs between the experimental and the control groups

The most common AEs included gastrointestinal disorders, hyperkalemia, nausea, dizziness, headache, infections, and infestations. The proportion of patients with one or more AEs\textsuperscript{18–20,22,23} was similar between the experimental and control groups. The $P$-value of the heterogeneity test was 0.71, so a fixed-effects model was chosen. The pooled mean difference was 1.16 (95% CI: 0.81–1.67). There was no difference in AEs between the two groups ($P=0.42$; Figure 5 and Table 2).

Severe AEs (SAEs) included vascular access complications, femoral neck fracture, noncardiac chest pain, and dyspnea. Four reports\textsuperscript{21–24} were included in this meta-analysis for patients with SAEs. The $P$-value of the heterogeneity test was 0.72, so a fixed-effects model was chosen. The pooled mean difference was 1.56 (95% CI: 0.91–2.66). The SAEs between the experimental and control groups were not statistically different ($P=0.11$; Figure 6 and Table 2).

## Discussion

HIF stabilizers promote erythropoiesis by stimulating endogenous EPO secretion via PHD inhibition.\textsuperscript{19,21} This meta-analysis showed that treatment with HIF stabilizers resulted in higher $\Delta$Hb levels in CKD patients. HIF stabilizers have been shown to effectively ameliorate anemia resulting from CKD. Based on the results of heterogeneity test ($P<0.00001$, $I^2=93\%$), we made a subanalysis of the NDD-CKD (HIF stabilizer group vs placebo group) and DD-CKD (HIF stabilizer group vs control group) subgroups. The pooled mean difference in the NDD-CKD subgroup was 3.51 (95% CI: 2.20–4.82, $P<0.00001$); however, the mean difference in the DD-CKD subgroup was 1.20 (95% CI: −0.12 to 2.51, $P=0.07$), although it was not statistically significant.
Iron metabolism indexes between the NDD-CKD and DD-CKD subgroups were found to be varied. The findings of the present study were discordant with those of earlier investigations, showing that HIF stabilizers correct anemia in DD-CKD patients. This discrepancy in findings may be attributable to three reasons. First, due to a moderate sample size and a short treatment period, we were unable to generate a robust conclusion on the effect of HIF stabilizers in DD-CKD patients. Second, anemia secondary to CKD is generally due to endogenous EPO deficiency, and desensitization of the oxygen-sensing mechanism may result in abnormally low EPO production under hypoxia. Third, HIF stabilizers are essentially less effective in DD-CKD than in NDD-CKD patients. Regardless of the mechanism of normal feedback control of erythropoiesis in CKD patients, stimulation of endogenous EPO production by HIF serves as a novel method for the treatment of anemia.

CKD anemia is a type of chronic inflammatory anemia, and hepcidin is induced in large quantities in infected and inflammatory states, resulting in intracellular iron retention and decreased plasma iron levels, eventually leading to anemia. PHIs induce erythropoiesis by improving the bioavailability and utilization of iron. Our meta-analysis indicated that in the NDD-CKD subgroup, Δferritin and Δhepcidin levels in the HIF stabilizer group were lower than in the placebo group. The HIF stabilizer group exhibited higher ΔTIBC compared to the placebo group. However, we did not find such changes in the DD-CKD subgroup. Our results on iron metabolism index in the DD-CKD subgroup were similar to those described by Holdstock et al. In their hemodialysis-dependent study, the authors hypothesized that the mean levels of hepcidin in patients who needed to receive rhEPO were suppressed before detection. Solak et al suggested that hepcidin gene suppression is not due to a direct effect of HIFs, but rather is secondary to erythropoiesis induced by EPO. Similarly, our analysis of the DD-CKD subgroup showed that there were no statistically significant differences in the ΔHb and Δhepcidin levels between the HIF stabilizer and control groups. However, it is also possible that we were unable to detect subtle differences due to our small sample size. Therefore, we plan to conduct further investigations to confirm the role of HIF stabilizers in hepcidin metabolism.

This study has some limitations, thereby requiring confirmation using additional studies. The chemical formulas of roxadustat, daprodustat, molidustat, and vadadustat are all different. However, no clinical trial to determine whether these have differential efficacies has been conducted to date. The sample size used in EPO measurements was relatively small, thereby preventing us from conducting meta-analysis for EPO levels. Furthermore, the present study was conducted for a short term, and thus, no long-term follow-up in terms of mortality, cardiovascular morbidity, and quality of life was performed.

Conclusion

In this meta-analysis, HIF stabilizers reduced ferritin and hepcidin levels, and increased Hb level and TIBC in NDD-CKD patients. However, HIF stabilizers only increased the TIBC, and did not affect the ferritin, hepcidin, and hemoglobin levels in DD-CKD patients. In summary, HIF stabilizers increase Hb levels and TIBC, and decrease the levels of ferritin and hepcidin in NDD-CKD patients, indicating that these are safe for short-term use in the treatment of anemia in NDD-CKD patients. However, the long-term safety and efficacy of HIF stabilizers in DD-CKD patients remain to be investigated.

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Disclosure

The authors report no conflicts of interest in this work.

References


