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CLINICAL RESEARCH

The Efficacy and Safety of Roxadustat for the Treatment of Posttransplantation Anemia: A Randomized Study

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Introduction: Roxadustat, an oral hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitor, can stimulate erythropoiesis. Our objective was to evaluate the efficacy and safety of roxadustat for the treatment of posttransplantation anemia (PTA).

Methods: A total of 150 adult renal transplant recipients who underwent PTA were randomized to either the experimental group or the control group. During the 12-week randomized phase, the experimental group was randomized to oral iron and roxadustat treatment, and the control group was randomized to oral iron treatment only. The randomized phase was followed by a 12-week extended treatment period in which all participants were prescribed roxadustat treatment according to hemoglobin (Hb) levels. All the participants were followed-up with every 4 weeks. The primary end points were the change in Hb levels and response rate throughout the randomized period.

Results: A total of 128 participants completed the randomized treatment period (90 in the experimental group and 38 in the control group). The mean Hb concentration at week 12 was 12.20 g/dl in the experimental group and 11.19 g/dl in the control group. A significantly higher proportion of participants who achieved Hb responses were in the experimental group than in the control group. Differences in serum iron, total iron-binding capacity (TIBC) and transferrin from baseline to week 8 to 12 were significant between the 2 groups. The adverse event profiles were comparable between the 2 groups.

Conclusion: Roxadustat increased Hb in adult renal transplant recipients who underwent PTA, with an adverse event profile comparable to that of the control group.

Kidney Int Rep (2024) **9,** 1705–1717; https://doi.org/10.1016/j.ekir.2024.04.021
KEYWORDS: chronic kidney disease; HIF signaling; randomized controlled study; renal anemia; renal transplantation; roxadustat

See Commentary on Page 1561

Renal anemia is one of the main complications of chronic kidney disease (CKD), which is associated with the prognosis of patients with CKD.[1] Severe anemia is associated with a high risk of mortality, cardiovascular events, and hospitalization.[2,3] Renal transplantation is the best form of renal replacement therapy for end-stage renal disease.[4] However, renal anemia after kidney transplantation, which is called PTA, is common.[5] The prevalence of PTA is highest at the time of renal transplantation. With the recovery of renal function, Hb levels increase. However, approximately 40% of patients who accept renal transplantation are still anemic at about 3 months after transplantation.[6] Many factors have been associated with PTA, including iron deficiency, worsening renal function, the use of immunosuppressive medications, the use of drugs acting on the renin angiotensin axis, infections, and abnormally low levels of erythropoietin (EPO).[7,8]

Erythropoiesis-stimulating agents (ESAs) are the standard therapy for renal anemia.[9] Many safety

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Received 1 July 2023; revised 1 April 2024; accepted 8 April 2024; published online 15 April 2024

concerns have emerged regarding the use of ESAs, including the occurrence of cardiovascular events and vascular access thrombosis.[10,11] Furthermore, ESAs are administered by injection but not oral administration, which is a potential obstacle for patients. Therefore, it is common for patients with PTA to remain undertreated due to concerns about the safety of ESAs and additional injections.[12] This condition will further affect graft function and graft survival.[7] Therefore, a number of pharmaceutical companies have attempted to identify alternative therapeutic approaches for treating renal anemia.

Hypoxia-inducible factor (HIF) is a key transcription factor that can regulate multidownstream genes. Under normoxia, HIF-PH is active and promotes the degradation of HIF-α. HIF-PH inhibitors can reversibly stabilize HIF-α and subsequently upregulate the transcription of HIF downstream genes. Some of the downstream genes can regulate the erythropoietic response, including those encoding endogenous EPO, enzymes involved in heme biosynthesis, and proteins that promote the availability of bone marrow iron.[13-15] Roxadustat is an HIF-PH inhibitor approved by the regulatory agency to treat anemia in patients with CKD in Europe, China, and Japan, but has not been approved in the United States to date.[16] Roxadustat significantly increased and maintained Hb levels in various randomized controlled studies in different cohorts, including nondialysis patients, patients on hemodialysis, and patients on peritoneal dialysis.[17-26]

With the increase in the number of patients who have undergone kidney transplantation in recent years, the population with PTA has also expanded. However, there is no randomized controlled trial on the use of roxadustat in patients with PTA. Compared to other patients with CKD, patients after renal transplantation have their own characteristics, such as immunosuppressive state and high risk of infection.[27,28] Here, we first carried out a randomized controlled study to evaluate the efficacy of roxadustat for PTA. Furthermore, we evaluated the safety of roxadustat in PTA, including its effect on renal function, influence on plasma tacrolimus concentration, risk of infection events and thrombosis events, and other adverse events.

METHODS

Trial Design and Oversight

This was a randomized, open-label study conducted at the First Affiliated Hospital, Zhejiang University of Medicine School, between October 2021 and August 2022.

A total of 150 participants were recruited from a single center, the First Affiliated Hospital, Zhejiang University of Medicine School. Eligible participants were aged between 18 and 75 years, had CKD, and received a kidney transplant more than 6 months before enrolment. None of the participants had received ESAs for at least 4 weeks before randomization. The participants had stable renal function in the last 3 months and no clinical evidence of acute or chronic rejection. During screening, Hb values were in the range of 9 g/dl to 11 g/dl. The details on the inclusion and exclusion criteria are provided in Supplementary Table S1.

The study comprised a screening period, a 12-week randomized treatment phase, and a 12-week extended treatment period. After the screening period, 150 eligible participants were randomized (1:2) into the control group or the experimental group. Randomization was performed centrally and stratified according to estimated glomerular filtration rate (eGFR) (<60 ml/min per 1.73 m^2 or ≥60 ml/min per 1.73 m^2) and Hb levels at screening (<10 g/dl or ≥10 g/dl). During the 12-week randomized treatment phase, the experimental group was randomized to oral iron and roxadustat treatment, and the control group was randomized to oral iron treatment only. For the extended treatment period, all participants who continued the trial were prescribed roxadustat according to their Hb level. During the whole course of the study, all the participants were followed-up with every 4 weeks in our hospital (Figure 1). Data on symptoms, physical examination findings, clinically significant laboratory results, and hospital visits during the last 4 weeks, were collected. The randomization, treatment, and follow-up were carried out by the clinical physician in our study team.

The Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University approved the research project (IIT20200117C-R1). Informed consent was signed by all participating participants. This study was performed in accordance with the ethical principles of the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice.

Treatment

The type of oral iron supplement used was limited to iron polysaccharide complex capsules (150 mg/capsule). The dose was a single daily dose of 3 mg/kg. Iron was adjusted according to the Chinese guideline to maintain transferrin saturation (TSAT) in the range of 30% to 50%.[29] The dose adjustment method of roxadustat was performed according to the manufacturer's instructions. The participants started roxadustat at a dose of 100 mg for oral administration 3 times a week. The dose of roxadustat was adjusted every 4 weeks to maintain a Hb level >12 g/dl. The does exposure for

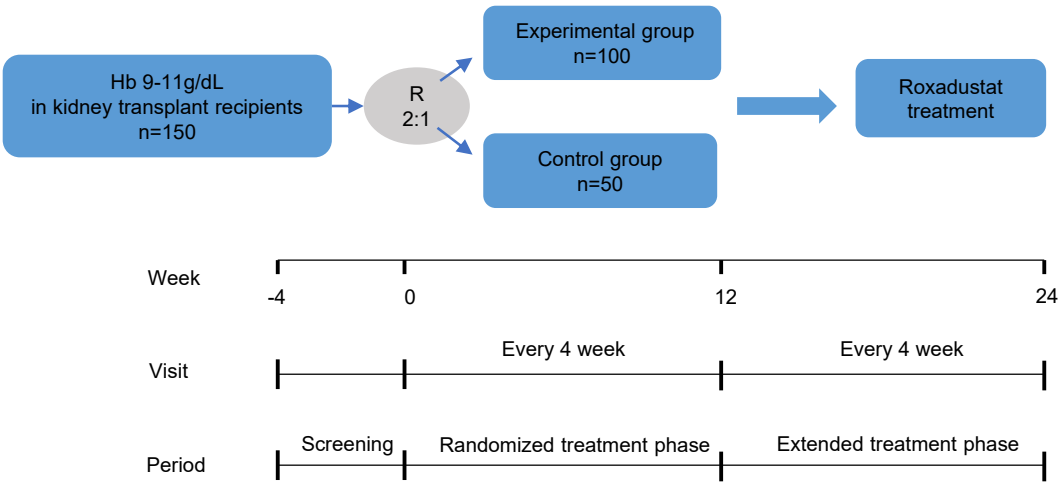

Figure 1. Design of the study. The study comprised a screening period, a 12-week randomized treatment phase, and a 12-week extended treatment period. After the screening period, 150 eligible patients were randomized (1:2) into the control group or the experimental group. During the 12-week randomized treatment phase, the control group was assigned to oral iron treatment for renal anemia, and the experimental group was assigned to roxadustat and oral iron treatment. For the extended treatment period, all patients who continued the trial received roxadustat treatment according to the hemoglobin level. During the course of the study, all patients were followed-up with every 4 weeks. Hb, hemoglobin.

our participants is shown in Supplementary Figure S1. Iron therapy using i.v. was prohibited except rescue therapy. Rescue therapy, which included blood transfusion, i.v. iron, the use of ESAs, or any combination of the 3 therapies, was offered to participants with a Hb level <8 g/dl and to those with a Hb level between 8 to 9 g/dl who had a decrease of Hb>1 g/dl from baseline. For immunosuppressive medications, maintenance immunosuppression after transplantation in the included participants consisted of prednisolone, mycophenolate mofetil, and tacrolimus. The dose was adjusted according to the Chinese guideline.[30]

End Points

The primary efficacy end points for the trial included the change in Hb levels from baseline to week 12 and the cumulative response rate of Hb (Hb level >12 g/dl and ΔHb from baseline >1 g/dl) during the 12-week randomized treatment period. The secondary end points included changes in iron metabolism indices, changes in serum lipid, and physical activity assessment.

Rapid Assessment of Physical Activity (RAPA) Score

The RAPA is a self-assessment tool that has been shown to be a good tool for measuring physical activity in a clinical setting. The questions in the 9-item questionnaire range from a sedentary lifestyle to regular and strenuous physical activity, and from strength training to flexibility-enhancing exercises. The RAPA questionnaire was obtained via the website: http://depts.washington.edu/hprc/rapa.[31]

Semiautomated Enzyme-Linked Immunosorbent Assay

The test for platelet activation markers was obtained informed and consent from 40 participants for these additional tests. The plasma was separated from whole blood by centrifugation at 4 °C at 1500*g* for 10 minutes and stored at −80 °C. The frozen plasma samples were assayed using enzyme-linked immunosorbent assay according to the manufacturer's instructions. Forty participants had access to platelet activation markers, including β-thromboglobulin (Novus USA) and P-selectin (R&D Systems Europe) at 2 time points (before treatment and after 8 consecutive weeks of roxadustat treatment).

Safety Assessments

Adverse events were monitored throughout the trial period. The frequency of safety assessment was at least once every 4 weeks. The evaluation of adverse events was performed according to the Common Terminology Criteria for Adverse Events, Version 5.0. Safety evaluation included stabilization of renal function (eGFR and serum creatinine), stabilization of blood tacrolimus concentrations, thrombotic risk (thrombotic events and markers of platelet activation), infection events, and other adverse events. We obtained information on adverse events at each visit, including, but not limited to, symptoms, clinically significant laboratory results, physical examination findings, and hospital visits during the last 4 weeks.

Statistical Analysis

PASS software (version 15, Kaysville, UT) was used to calculate the sample size. Statistical analysis was

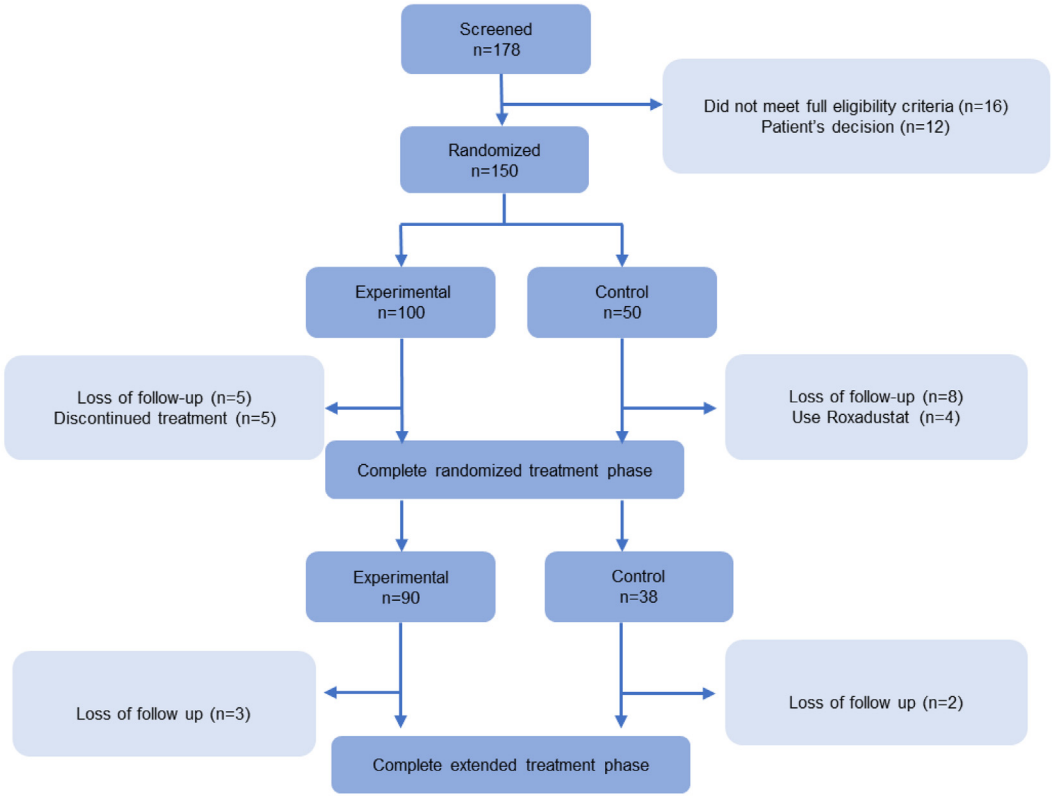

Figure 2. Patient disposition.

performed with the assistance of R software (version 4.0.2; http://www.R-project.org) and SPSS 26.0 software (SPSS Inc., Chicago, IL).

According to the literature, we estimated the number of enrolled participants with ≥90% power to detect at least a 1 g/dl difference in Hb between the groups, assuming an SD of 1.5 and $\alpha = 0.05$. Considering potential dropouts, 150 participants were enrolled.

Continuous variables are expressed as the mean ± SD, and categorical variables are described as numbers and percentages. When the data fit the normal distribution, an independent 2-sample *t* test was used to compare the means between the groups. When the data did not conform to a normal distribution, the Mann-Whitney *U* test was used for continuous variables between groups. The nominal variables were analyzed using the chi-square test. Two-way analysis of variance was performed for comparisons at different time points.

To compare the primary and secondary end points, we used a mixed-effects repeated measures model that included data from all visits during the randomized phase, with adjustment covariates that included trial agent, trial visit, baseline eGFR, and hypertension history. The adjustment covariates were in reference to a previous study.[18] We used the mixed-effects repeated measures model to calculate *P*-value and 2-sided 95% confidence interval (CI) for the difference in least-squares mean (LSM). When more than 5% of values were missing for a variable, the missing data was handled using multiple imputations in a sensitivity analysis. A 2-tailed *P*-value < 0.05 was considered to indicate statistical significance in all the statistical analyses.

RESULTS

Participants Characteristics

From October 2021 to August 2022, a total of 150 participants (100 in the experimental group and 50 in the control group) underwent randomization. During the 12-week randomized treatment period, 9 did not receive a trial regimen and 13 were lost to follow-up, leaving a population of 128. A total of 128 participants completed the 12-week randomized treatment period (90 in the experimental group and 38 in the control group) (Figure 2). The baseline characteristics were similar between the 2 groups (Table 1).

End Points

During the randomized treatment phase, the mean change in the Hb concentration from baseline to week 12 was 1.76 ± 1.36 g/dl in the experimental group and 0.69 ± 0.97 g/dl in the control group. The difference in LSM change was significantly greater in the experimental group than in the control group at 4, 8, and 12 weeks of the randomized treatment phase (Table 2). The Hb levels

Table 1. Characteristics of the patients at baseline

Characteristics	Control group (n = 38)	Experimental group (n = 90)	P-value
Age, yr	46.08 ± 11.18	41.96 ± 10.90	0.06
Male, n (%)	8 (21.1)	19 (21.1)	0.99
BMI, kg/m^2	21.65 ± 3.30	21.22 ± 3.29	0.51
History of diabetes mellitus, n (%)	5 (13.2)	6 (6.7)	0.41
History of hypertension, n (%)	25 (65.8)	53 (58.9)	0.51
Time from CKD diagnosis to transplantation, yr	7.09 ± 6.82	7.67 ± 7.08	0.67
Time for dialysis, yr	3.14 ± 4.19	2.61 ± 2.80	0.41
Time after kidney transplantation, yr	8.13 ± 6.68	6.02 ± 5.68	0.09
Hemoglobin			
Mean, g/dl	10.56 ± 0.44	10.43 ± 0.59	0.22
<10 g/dl, n (%)	6 (15.8)	22 (24.4)	0.28
≥10 g/dl, n (%)	32 (84.2)	68 (75.6)	-
eGFR			
Mean, ml/min per 1.73 m^2	50.19 ± 20.03	53.22 ± 21.09	0.45
>60 ml/min per 1.73 m^2, n (%)	11 (28.9)	32 (35.6)	0.39
30–60 ml/min per 1.73 m^2, n (%)	20 (52.6)	49 (54.4)	-
<30 ml/min per 1.73 m^2, n (%)	7 (18.4)	9 (10)	-
Oral iron supplementation, n (%)	20 (52.6)	50 (55.6)	0.88
Iron, ug/l	15.12 ± 5.14	12.51 ± 7.22	0.05
Immunosuppression dose, mg/d			
Tacrolimus	4.00 ± 1.01	4.20 ± 1.13	0.35
Mycophenolic acid	903.68 ± 241.56	946.89 ± 263.94	0.39
Steroids	7.11 ± 2.50	6.68 ± 2.63	0.52

BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.
Values are expressed as mean ± SD and n (%).

of the experimental group at 4, 8, and 12 weeks were significantly greater than those at baseline ($P < 0.01$); however, the Hb levels of the control group at 4, 8, and 12 weeks were not significantly different from those at baseline (Figure 3a). Similarly, a significantly greater proportion of participants in the experimental group (78.89%) achieved an Hb response (ΔHb from baseline > 1 g/dl) compared to the control group (45.95%) (relative risk 1.72; 95% CI, 1.25–2.57; $P < 0.01$). The Hb response (Hb > 12 g/dl during the random phase) was also more common in the experimental group (63.33%) than in the control group (34.21%) (relative risk 1.85; 95% CI, 1.21–3.05; $P < 0.01$) (Figure 3b).

Table 2. Statistical analysis of change in hemoglobin levels from baseline to the weeks 4 to 12

Items	Control group (n = 38)	Experimental group (n = 90)
Hb at BL, g/dl	10.56 ± 0.44	10.43 ± 0.59
Hb at week 4, g/dl	10.74 ± 0.86	11.55 ± 1.20
Hb change from BL to week 4, g/dl	0.21 ± 0.81	1.12 ± 1.11
LSM difference (95% CI)	0.92 (0.48–1.36)	
P-value	< 0.01	
Hb at week 8, g/dl	11.28 ± 1.26	12.18 ± 1.35
Hb change from BL to week 8, g/dl	0.72 ± 1.21	1.75 ± 1.40
LSM difference (95% CI)	1.01 (0.54–1.47)	
P-value	< 0.01	
Hb at week 12, g/dl	11.19 ± 0.94	12.20 ± 1.25
Hb change from BL to week 12, g/dl	0.69 ± 0.97	1.76 ± 1.36
LSM difference (95% CI)	1.04 (0.58–1.51)	
P-value	< 0.01	

BL, baseline; CI, confidence interval; Hb, Hemoglobin; LSM, least-squares mean.
± values are means ± SD.

Iron Metabolism Changes

During the randomized treatment phase, plasma iron concentration increased in the experimental group but not in the control group. The difference in iron LSM between the experimental group and the control group was not significant at week 4 (LSM difference, 2.51 g/dl; 95% CI, −1.79 to 6.81; $P = 0.25$) but was significant at week 8 to 12 (LSM difference, 4.76 g/dl; 95% CI, 1.07–8.46; $P = 0.01$). Furthermore, TIBC and transferrin showed similar increases in the experimental group, but not in the control group. The LSMs for transferrin and TIBC were significantly different between the experimental group and the control group at week 4 and week 8 to 12 (Table 3). The change in TSAT was not significant between the experimental group and the control group.

Blood Lipid Changes

Participants in the experimental group showed decreases in blood lipid indices, including total cholesterol, triglycerides, low-density lipoprotein cholesterol, and

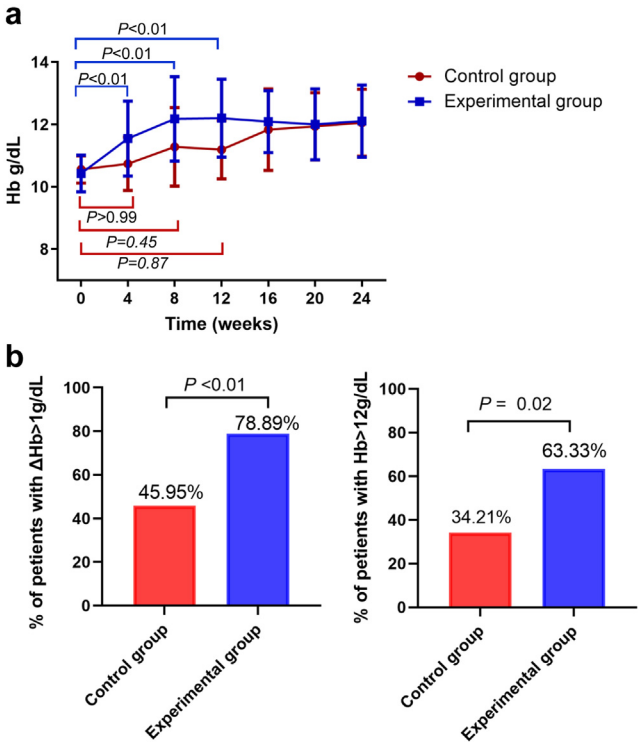

Figure 3. Changes in hemoglobin concentrations. (a) Mean hemoglobin levels over time in the experimental group and the control group; (b) cumulative response rate (ΔHb from baseline >1 g/dl and Hb level >12 g/dl) in the experimental group and the control group. Hb, hemoglobin.

Table 3. Statistical analysis of change in iron metabolism from baseline to week 4 and weeks 8 to 12

Items	Iron (μmol/l)	Transferrin (mg/dl)	TIBC (μmol/l)	TSAT (%)
BL				
Control group	15.12 ± 5.14	238.88 ± 36.62	53.25 ± 10.12	30.54 ± 16.46
Experimental group	12.51 ± 7.22	236.61 ± 45.85	49.92 ± 11.27	27.19 ± 17.22
Week 4				
Control group	13.68 ± 5.25	208.46 ± 32.55	48.81 ± 8.68	29.28 ± 13.73
Experimental group	13.58 ± 8.95	285.11 ± 63.98	62.72 ± 13.90	23.15 ± 16.71
Difference in change from BL				
LSM difference (95% CI)	2.51 (−1.79 to 6.81)	78.92 (58.69–99.15)	17.24 (12.64–21.83)	−2.78 (−11.14 to 5.60)
P-value	0.25	< 0.01	< 0.01	0.51
Week 8–12				
Control group	13.55 ± 5.09	227.38 ± 40.29	51.20 ± 8.93	26.42 ± 10.58
Experimental group	15.71 ± 7.56	275.78 ± 61.54	59.93 ± 12.28	24.72 ± 13.89
Difference in change from BL				
LSM difference (95% CI)	4.76 (1.07–8.46)	50.68 (26.82–74.54)	12.05 (6.37–17.74)	1.64 (−6.72 to 10.0)
P-value	0.01	< 0.01	< 0.01	0.70

BL, baseline; CI, confidence interval; LSM, least-squares mean, TSAT, transferrin saturation, TIBC, total iron-binding capacity.
± values are means ± SD.
$n = 26$ in the control group and $n = 74$ in the experimental group.

high-density lipoprotein cholesterol. The differences in LSM changes of the above 4 indices between the experimental group and the control group were significant at week 4 of the randomized treatment phase. However, the difference in LSM change from baseline to week 12 between the experimental group and the control group was significant only for high-density lipoprotein cholesterol but not for total cholesterol, triglycerides, or low-density lipoprotein cholesterol (Table 4).

Changes in Physical Activity

Physical activity can improve with the remission of renal anemia. Thus, we also evaluated the change in physical activity by RAPA. However, there was no difference in RAPA between the control group and the experimental group from baseline to week 12 (Supplementary Figure S2).

Safety and Adverse Events

During the randomized phase, at least 1 adverse event was reported in 15 of 90 participants (16.67%) in the experimental group and 8 of 38 participants (21.05%) in the control group. The frequency of adverse events did not differ between the experimental group and the control group. Common adverse events included infection, increased serum creatine, and diarrhea. There were no deaths during the entire trial. Three participants (2 participants in the experimental group and 1 participant in the control group) had severe anemia and accepted rescue therapy. These 3 participants with severe anemia were comorbid with systemic

Table 4. Statistical analysis of change in blood lipid from baseline to week 4 and week 12

Items	Total cholesterol (mmol/l)	Triglycerides (mmol/l)	HDL (mmol/l)	LDL (mmol/l)
Baseline				
Control group	4.65 ± 1.17	1.43 ± 0.64	1.57 ± 0.44	2.47 ± 0.85
Experimental group	4.89 ± 1.11	1.45 ± 0.66	1.67 ± 0.55	2.58 ± 0.80
Week 4				
Control group	4.93 ± 1.36	1.74 ± 1.35	1.65 ± 0.50	2.60 ± 1.00
Experimental group	4.03 ± 0.98	1.41 ± 0.70	1.35 ± 0.40	2.05 ± 0.68
Difference in change from BL				
LSM difference (95%CI)	−1.11 (−1.46 to −0.77)	−0.37 (−0.62 to −0.13)	−0.40 (−0.57 to −0.24)	−0.64 (−0.88 to −0.40)
P-value	< 0.01	< 0.01	< 0.01	< 0.01
Week 12				
Control group	4.71 ± 0.93	1.49 ± 0.57	1.79 ± 0.49	2.40 ± 0.67
Experimental group	4.67 ± 1.09	1.46 ± 0.73	1.58 ± 0.49	2.42 ± 0.78
Difference in change from BL				
LSM difference (95% CI)	−0.16 (−0.53 to 0.20)	−0.04 (−0.30 to 0.22)	−0.25 (−0.42 to −0.07)	−0.02 (−0.27 to 0.24)
P-value	0.38	0.76	0.01	0.90

BL, baseline; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; LSM, least-squares mean; CI, confidence interval.
± values are means ± SD.
$n = 38$ in the control group and $n = 90$ in the experimental group.

Table 5. Summary of adverse events

Preferred term	Control group (n = 38)	Experimental group (n = 90)
Subjects with adverse event	8 (21)	15 (17)
Diarrhea	2 (5)	4 (4)
Edema	1 (3)	1 (1)
Headache	1 (3)	2 (2)
Insomnia	1 (3)	2 (2)
Renal impairment		
Rejection	1 (3)	0 (0)
Drug related renal injury	1 (3)	0 (0)
Acute tubulointerstitial nephritis	0 (0)	1 (1)
Prerenal acute renal injury	1 (3)	1 (1)
Infection related renal injury	1 (3)	2 (2)
Infection		
Lung	1 (3)	2 (2)
Intestinal system	1 (3)	1 (1)
Intracranial	1 (3)	1 (1)
Urinary tract	0 (0)	1 (1)
Cancer	0 (0)	1 (1)
Accept rescue therapy	1 (3)	2 (2)

Values are presented as n (%).

diseases such as cancer and infection. Two participants (1 in the experimental group and 1 in the control group) had severe systemic infection, and the Hb level of these participants suddenly decreased as the infection progressed. One patient in the experimental group was diagnosed with rectal cancer during the trial, and the Hb level decreased after surgery (Table 5).

The average eGFR did not differ significantly between the experimental group and the control group at different time points during the follow-up period (Supplementary Figure S3). The change in eGFR from baseline to week 12 was slight both in the experimental group and in the control group (control group: −1.47 ± 7.78 ml/min per 1.73 m^2; experimental group: 0.17 ± 9.20 ml/min per 1.73 m^2). The difference in LSM for the change in eGFR between the experimental group and the control group was small and insignificant (1.61 ml/min per 1.73 m^2; 95% CI, −2.15 to 5.38; P = 0.40) (Table 6). For transplant recipients, maintaining stable tacrolimus concentrations is crucial. In addition, we tested the change in tacrolimus blood concentrations before treatment and after 4 weeks of treatment. Between these 2 time points, there was no dose adjustment for the tacrolimus dose. The change in tacrolimus blood concentrations was small in both the experimental group and the control group. There was also no difference in LSM between the experimental group and the control group (0.74 ng/ml; 95% CI, −0.20 to 1.68; P = 0.12) (Table 7).

No serious adverse thrombotic events or thromboembolic events occurred throughout the trial. We also collected plasma from 40 participants at 2 time points, before roxadustat treatment and after 8 consecutive weeks of roxadustat treatment. There was no significant change in the levels of the platelet activation markers (including P-selectin and β-thromboglobulin) between before and after 8 weeks of connective roxadustat treatment (Figure 4).

Table 6. Statistical analysis of change in renal function from baseline to week 12

Items	Control group (n = 38)	Experimental group (n = 90)
eGFR at BL	50.19 ± 20.03	53.22 ± 21.09
eGFR at week 12	47.24 ± 18.44	52.51 ± 21.83
eGFR change from BL to week 12	−1.47 ± 7.78	0.17 ± 9.20
LSM difference (95% CI)	1.61 (−2.15 to 5.38)	
P-value	0.40	

BL, baseline; CI, confidence interval; eGFR, estimated glomerular filtration rate; LSM, least-squares mean.
± values are means ± SD.
The units for eGFR is ml/min per 1.73 m^2.

Extended Treatment Phase

After the initial randomized phase of the trial, 87 participants in the experimental group and 36 participants in the control group entered the extended treatment period, during which all participants received roxadustat according to their Hb level. Among the participants who were initially randomized to roxadustat, Hb levels remained stable during the extended treatment period. In the control group, the mean Hb level increased to a level comparable to that in the experimental group at the end of the extended treatment period (Figure 3a).

DISCUSSION

This clinical trial is the first randomized controlled trial to specifically evaluate the efficacy and safety of roxadustat for PTA. Our results suggested that the administration of oral roxadustat in patients with PTA is effective and safe. The primary efficacy analysis showed a significant increase in Hb level over the 12-week randomized treatment period in the experimental group. Similarly, Hb responses (ΔHb >1 g/dl from baseline; Hb > 12 g/dl) were higher in the experimental group than in the control group. Renal

Table 7. Statistical analysis of change in plasma tacrolimus concentration from baseline to week 4

Items	Control group (n = 38)	Experimental group (n = 90)
Tacrolimus at BL, ng/ml	5.44 ± 1.05	5.67 ± 0.99
Tacrolimus at week 4, ng/ml	6.17 ± 2.21	7.12 ± 1.93
Tacrolimus change from BL to week 4, ng/ml	0.61 ± 1.99	1.46 ± 2.13
LSM difference (95% CI)	0.74 (−0.20 to 1.68)	
P-value	0.12	

BL, baseline; CI, confidence interval; LSM, least-squares mean.
± values are means ± SD.

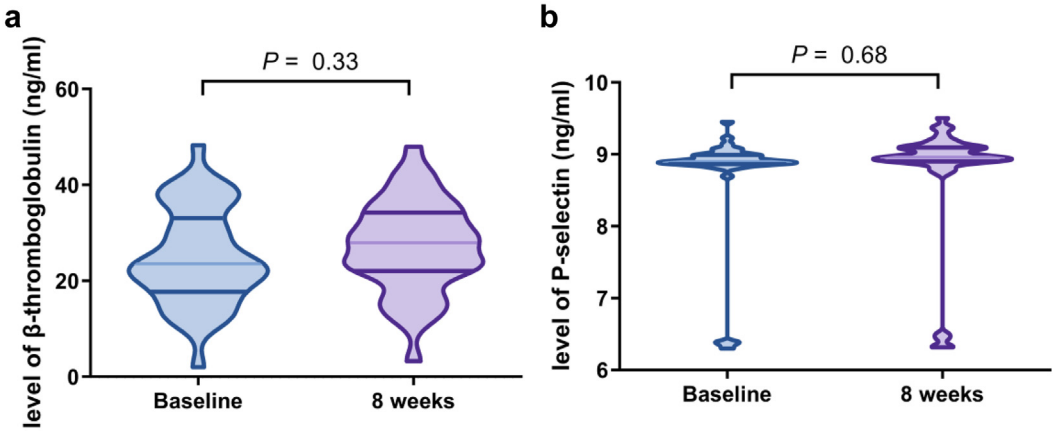

Figure 4. Statistical analysis of the change in platelet activation markers after 8 continuous weeks of roxadustat treatment. (a) P-selectin; (b) β-thromboglobulin. $n = 40$ for each group.

function was stable throughout the trial in both groups and the adverse event profiles were comparable between the experimental group and the control group.

PTA is common among kidney transplant patients, because different studies have reported a prevalence of 20% to 51% at various time points after transplantation.[32] The pathogenesis of PTA is complicated. Like anemia in CKD, iron deficiency and impaired EPO production also play a role in PTA. In addition, immunosuppressive drugs, antiviral agents, and angiotensin-converting enzyme inhibitors can suppress bone marrow and consequently cause anemia. In addition, viral infections can cause hematological abnormalities that result in anemia.[5,33] Increased hepcidin could also contribute to PTA and resistance to ESA treatment.[34] PTA has been shown to be negatively associated with higher rates of all-cause mortality, graft failure, and congestive heart failure.[35–38] Despite its frequent appearance and association with adverse long-term outcomes, there are no specific guidelines for Hb targets or therapeutic options for anemia after renal transplantation. With renal transplantation increasing in recent years, the population with PTA has also expanded. Thus, PTA treatment is crucial in the management of renal transplantation patients.

Roxadustat is an oral HIF-PH inhibitor that stabilizes HIF-α subunits (including HIF-1α and HIF-2α), maintains their activity, and activates downstream genes of HIF signaling.[39] Because of the numerous downstream pathways involved, roxadustat may have multiple effects on various biological and physiological processes. EPO is in the downstream pathway of HIF signaling, which can be activated by roxadustat. Circulating EPO then binds to the EPO receptor on red cell progenitors in the bone marrow, causing red cell mass to increase. Roxadustat reduces hepcidin levels, promotes iron release from intestinal cells into the blood and iron absorption, and improves iron transport.[14,40,41] Based upon the above mechanism, roxadustat has been used for the treatment of renal anemia. Compared to traditional treatment with ESAs, roxadustat has shown noninferior treatment efficacy for renal anemia in patients with CKD according to multiple studies.[17,23,42] Because roxadustat appears to be effective and well-tolerated in anemic patients with CKD, we first carried out a randomized controlled study to determine the efficacy of roxadustat in patients with PTA. PTA can be divided into 2 types, namely early PTA and late PTA, according to the time after renal transplantation.[7] All the recipients enrolled in our study underwent transplantation more than half a year ago, thus, this study focused mainly on late PTA. This study met the primary objective of demonstrating the effective use of roxadustat in PTA.

Although our study revealed that roxadustat is an effective treatment for PTA, similar to previous studies in patients with CKD, we believe that the mechanism of roxadustat in PTA treatment is not completely the same. EPO deficiency and/or resistance have been implicated in the development of renal anemia. However, for PTA, the mechanisms of EPO deficiency and resistance have their own characteristics. In renal transplantation recipients, EPO resistance can be attributed partly to the inflammatory status induced by surgical intervention and the occurrence of infectious complications. EPO can be secreted from the proximal tubules, and renal tubular injury can decrease EPO secretion. For renal transplantation patients, delayed graft function, allograft rejection, and calcineurin inhibitor toxicity all can induce renal tubular injury, which then leads to decreased EPO secretion in tubules.[43] Treatment with roxadustat can help to relieve inflammation and renal tubular injury,[44] which may be one of the potential mechanisms underlying the effectiveness of roxadustat in treating PTA.

Iron deficiency is another cause of renal anemia. Iron deficiency can be divided into absolute iron deficiency and functional iron deficiency. Absolute iron deficiency occurs when the amount of stored iron in the liver, spleen, and marrow is minimal. However, functional iron deficiency can develop when erythropoiesis is enhanced by EPO or when iron release from storage is limited due to inflammatory processes in the presence of adequate or even increasing iron stores.[45] Both of these 2 types of iron deficiency can be found in PTA. Iron supplementation can ameliorate absolute iron deficiency, which is why we observed increased Hb in the control group. However, iron supplementation has difficulty correcting functional iron deficiency. As a previous study reported, roxadustat plays a regulatory role in iron metabolism and can further correct functional iron deficiency. Animal studies have shown that HIF plays an important role in the regulation of the transcription of divalent metal transporter 1, duodenal cytochrome b reductase 1, ferroportin, transferrin, and transferrin receptor.[46] On the basis of the above foundlings, roxadustat has its advantage in alleviating functional iron deficiency.

In our trial, serum iron concentration significantly increased after approximately 8 to 12 weeks of roxadustat treatment. Before the change in iron, transferrin and TIBC significantly increased as early as 4 weeks after roxadustat treatment. We hypothesized that the time difference between iron and transferrin or TIBC is related to the mechanisms by which HIFs affect anemia. HIFs can directly induce the transcription of transferrin, because transferrin contains 2 HIF binding sites in its 5′ enhancer region.[47] HIFs primarily induce the transcription of transferrin and then improve iron transport to tissues, which is accompanied by an increase in TIBC. Increased transferrin and TIBC ultimately led to improved iron metabolism and erythrocyte development.[48] By increasing iron utilization and absorption, roxadustat can reduce the safety risks of iron overload, oxidative stress, and hypersensitivity reactions.[49] Interestingly, TSAT decreased in both groups. One previous study also showed that roxadustat and ESAs both led to a decrease in TSAT, but their implications are different. The reduction in TSAT after roxadustat treatment may be associated with increased transferrin levels. However, the reduced TSAT levels after ESA treatment are primarily driven by reduced serum iron levels, reflecting an imbalance between iron consumption and availability.[50] Hepcidin is another key regulator of iron absorption and mobilization from hepatocytes and macrophages. Hepcidin can be downregulated under conditions of hypoxia or HIF stabilization, and low hepcidin levels contribute to the increased synthesis of iron transport proteins and enhanced intestinal absorption of iron.[51] Previous studies have shown that serum hepcidin levels are reduced in patients receiving roxadustat treatment, but hepcidin was not tested in our study.

The safety of roxadustat was also analyzed in our trial. The results showed that the proportion of participants with 1 or more adverse events was comparable between the experimental group and the control group. In our study, the most frequently reported adverse event was infection. A meta-analysis showed that the proportion of patients with 1 or more serious adverse events in the roxadustat group was comparable to that in the control group (placebo or epoetin alfa) in the cohort of nondialysis-dependent patients and dialysis-dependent patients with CKD, but the incidence of some adverse events (such as hyperkalemia) in the roxadustat group was significantly greater than that in the control group.[17,18,23] Hyperkalemia did not occur in our trial because the renal transplant patients included in our trial had better renal function than those included in previous trials. It should be noted that the occurrence of adverse events is not directly associated with the administration of roxadustat. The differences in the types of adverse events reported in different studies are related to the characteristics of the included population. The most frequently reported adverse event in transplantation patients was infection due to immunosuppressive treatment. The most frequently reported adverse event in the dialysis-dependent cohort was hyperkalemia because of the low level of renal function.

The influence of roxadustat on platelet activation and thrombosis formation has been explored in previous studies. Robert Provenzano's study showed that ESA treatment can increase platelet count, but treatment with roxadustat showed stable platelet counts.[52] In addition, Jiaxin Zhao's team first evaluated the role of roxadustat in platelet activation both *in vivo* and *in vitro*. They found that roxadustat cannot stimulate platelet production (megakaryocyte maturation and proplatelet formation), platelet aggregation, spreading, or clot retraction; and did not increase the level of a platelet activation marker (P-selectin).[53] It was hypothesized that roxadustat can minimize iron deficiency–mediated reactive thrombocytosis by improving iron metabolic state, but the detailed mechanism needs additional exploration.[52] Consistent with the previous study, our study found that the risk of thrombosis in the experimental group was comparable to that in the control group. No thrombosis events occurred throughout the trial in both groups. Roxadustat treatment slightly increased platelet activation, but the level of platelet activation marker showed no significant differences between the 2 groups in our trial.

Roxadustat was first approved for the treatment of CKD anemia in China in December 2018, followed by Japan in 2019, and Europe in 2020. However, to date, this drug has not received marketing authorization in the United States. The US Food and Drug Administration rejected the approval application in July 2021 on the basis of concerns that it did not meet the prespecified noninferiority criteria for cardiovascular safety.[54] A pooled analysis evaluated the data from 3 phase 3, double-blind, and placebo control studies in nondialysis-dependent CKD, and found that roxadustat demonstrated noninferior cardiovascular safety to placebo. The hazard ratios for major adverse cardiovascular eevent and major adverse cardiovascular event-cause mortality met the reference noninferiority risk margin.[55] However, the safety of roxadustat in dialysis-dependent CKD was controversial. The initial report suggested that roxadustat was superior in terms of major adverse cardiovascular event. However, the conclusion was altered in an on-drug analysis, which showed that patients on roxadustat treatment actually had a higher cardiovascular risk than the company had originally reported. Similarly, the published meta-analysis also showed varied conclusions in terms of the incidence of adverse reactions of roxadustat in dialysis-dependent patients with CKD.[56–58] The safety factor was not limited to the data in random control studies, but also existed in its mechanism. Because roxadustat can upregulate the expression of a large number of transcriptional targets, there have been a number of theoretical concerns about this new class of drugs. These include, but are not limited to, increased angiogenesis, increased cancer risk, increased hypothyroidism, worsening renal progression due to kidney fibrosis, and exacerbation of pulmonary arterial hypertension.[59] Most of these concerns have not been adequately addressed in current published clinical trials. More data will certainly be required to assess its safety not only from further clinical trials, but also from postmarketing pharmacovigilance data.

This study has several limitations. Due to its design, this study is an open-label, placebo-free control trial. The lack of a placebo group may limit the interpretation of the efficacy and safety findings. The open-label design may have bias. Furthermore, this was a single-center study in China, and the baseline characteristics of the included participants have their own characteristics. Like the previous Chinese transplantation cohort,[8] our study revealed a low percentage of participants with diabetes and a low body mass index compared to those in Western countries. The differences in body mass index and diabetes incidence may be related to race, gender (male/female), and dietary habits. There was a large proportion of female participants in our trial, and we did not deliberately include female participants. On the basis of these results, we reviewed the literature and identified 2 potential explanations. As a previous study reported in China, the proportion of female patients in the PTA cohort increased,[6] which may be due to strict diet control and menstruation. The cut-off level for anemia in men is higher than that in women. During the screening period, some male patients with anemia did not meet the comparatively low criteria of our study (10–11 g/l). In addition, the relatively good renal function of our participants may make the conclusions derived from current studies inappropriate for other PTA groups. For iron metabolism, only serum iron, TIBC, and transferrin were measured; serum hepcidin was not measured.

In summary, our study demonstrated that roxadustat effectively improved anemia in patients with PTA and did not influence the risk of infection, renal function, the risk of thrombosis events, or plasma tacrolimus concentration, with an adverse event profile comparable to that of the control.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

Funding for this study was from the National Natural Foundation of China 82000635 and the Natural Foundation of Zhejiang LQ21H050004 (for WK), the National Natural Foundation of China U21A20350 (for JC), and the National Natural Foundation of China 82070767 (HH).

The clinical trial registry name and registration number were "Evaluation of the Therapeutic Value and Safety of HIF-PH Inhibitor in Renal Anemia Patients After Renal Transplantation, ChiCTR2100045309; and the registry date was April 10, 2021.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

Research idea and study design was by WK, XW, HH, and YL. Data acquisition was by MW, XLiu, XLin, ZS, and YQ. Data analysis or interpretation was by WK and XW. Statistical analysis was by WK and XW. Supervision or mentorship was by HH, JC, and HJ. HH and YL take responsibility that this study has been reported honestly, accurately, and transparently; and accept accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Roxadustat exposure in the experimental group.

Figure S2. Changes in physical activity.

Figure S3. Changes in renal function.

Table S1. Details regarding the inclusion and exclusion criteria.

File S1. Reporting checklist for randomized trials based on the CONSORT guidelines.

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