CORRESPONDENCE

Roxadustat for Anemia in Patients with Chronic Kidney Disease

TO THE EDITOR: Chen et al. (Sept. 12 issue)[1] report that in their trial of roxadustat (FGCL-4592-808) in patients with chronic kidney disease who were not undergoing dialysis, roxadustat was more effective than placebo in correcting anemia. In this trial, the authors underestimated the effect of the drug on iron metabolism and instead focused on serum iron levels. The frequency of a transferrin saturation of less than 20% or a ferritin level of less than 100 μg per liter is important for the clinical diagnosis of iron deficiency, but those values were reported only at baseline. Given the data on the ferritin level and transferrin saturation shown in this trial and in the companion trial (FGCL-4592-806) conducted by Chen et al. (Sept. 12 issue)[2] involving patients who were undergoing long-term dialysis, we must conclude that the development of iron deficiency is common with roxadustat.

In addition, the true effect of roxadustat on the development of hyperkalemia is impossible to understand. In the FGCL-4592-808 trial, hyperkalemia occurred in 16% of the patients who received roxadustat and in 8% of those who received placebo. In the FGCL-4592-806 trial, hyperkalemia occurred in 7% of the patients who received roxadustat and in 1% of the patients who received epoetin alfa. Without providing details, the authors stated that in that trial, reporting standards for hyperkalemia differed among trial sites. The article on the FGCL-4592-808 trial did not mention the criteria for hyperkalemia in adverse-event reporting, and data on hyperkalemia in Table S4 in the Supplementary Appendix (available with the full text of the article at NEJM.org) were restricted to patients in the 18-week open-label extension study.

Clinical guidance on how to address these two possibly important effects of roxadustat therapy should be provided by the investigators in order to avoid problems that have been seen with other agents (e.g., when spironolactone was introduced in clinical practice).[3]

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TO THE EDITOR: In the FGCL-4592-808 trial involving patients with chronic kidney disease who were not undergoing dialysis, Chen et al. report the correction and maintenance of a mean hemoglobin level in the roxadustat group. The authors report that the incidence of metabolic acidosis was six times as high and the incidence of hyperkalemia was twice as high in the roxadustat group as in the placebo group. We have conducted some cell-culture studies that may explain this phenomenon. Roxadustat and other hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors induce hypoxia-like conditions within the cell by stabilizing HIFs. The most pronounced effect of the action of HIFs is a transcriptionally mediated switch from aerobic to anaerobic metabolism. The consequence of the resulting increased glycolysis is tissue acidification associated with overproduction of lactic acid. Acidosis entails the release of potassium ions from cells, which then induces hyperkalemia.

Table 1. Expression of Glycolytic Genes in Human Mesenchymal Stromal Cells Cultured with Vadadustat or Incubated in 2% Oxygen.*

Gene Symbol, Ensembl Identifier, and Gene Name	Hypoxia		Vadadustat	
	Mean Change in Expression	Adjusted P Value	Mean Change in Expression	Adjusted P Value
ALDOA (ENSG00000149925; aldolase, fructose-bisphosphate A)	1.41	3.3×10^{-4}	1.40	2.4×10^{-2}
ALDOC (ENSG00000109107, fructose-bisphosphate aldolase C)	2.51	1.30×10^{-7}	2.66	2.4×10^{-2}
ENO2 (ENSG00000111674, gamma-enolase)	3.87	8.7×10^{-29}	2.88	5.2×10^{-4}
GPI (ENSG00000105220, glucose-6-phosphate isomerase)	1.51	5.8×10^{-7}	1.36	2.0×10^{-2}
LDHA (ENSG00000134333, lactate dehydrogenase A)	1.90	9.2×10^{-15}	1.52	2.5×10^{-2}
PFKL (ENSG00000141959, phosphofructokinase–liver type)	1.44	8.3×10^{-6}	1.31	4.3×10^{-2}
PGK1 (ENSG00000102144, phosphoglycerate kinase 1)	2.09	9.8×10^{-20}	1.79	7.2×10^{-4}
TPI1 (ENSG00000111669, triosephosphate isomerase 1)	1.59	3.7×10^{-9}	1.40	1.9×10^{-2}

* Listed are the RNA-sequencing results of glycolysis-related gene expression obtained from the functional enrichment analysis of differentially expressed genes (performed with the use of the DAVID database, version 6.8) in human bone marrow mesenchymal stromal cells obtained from six donors and cultured ex vivo with vadadustat (40 μmol per liter) or incubated in 2% oxygen (hypoxia) for 6 hours. Whole-exome sequencing was performed on the HiSeq 1500 platform with the use of the TruSeq Stranded mRNA Library Prep Kit (Illumina). The relative expression of transcripts was quantified for each donor with the use of Salmon software.[1] Files were mapped to the Homo_sapiens.GRCh38.95.gtf reference genome with the use of HISAT2 software, version 2.1.0.[2] The tximport pipeline was used to import transcript abundance data sets for the differential gene-expression analysis ($P<0.05$) with DESeq2 software, version 1.14.1.[3] The results are presented as changes in gene expression in treated cells as compared with mesenchymal stromal cells cultured in standard growth medium and at an atmospheric oxygen level; the P value was adjusted for multiple hypothesis testing with the false-discovery-rate method of Benjamini and Hochberg.

We performed RNA sequencing of human mesenchymal stromal cells which revealed that vadadustat (Akebia) at a concentration of 40 μmol per liter up-regulated expression of key glycolytic genes in a manner and magnitude similar to those induced by hypoxia (at 2% oxygen) (Table 1). Given the long-term adverse effects of metabolic acidosis, the risk profile associated with the use of HIF prolyl hydroxylase inhibitors in patients with chronic kidney disease warrants further definition.

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TO THE EDITOR: Chen et al. report that roxadustat, a prolyl hydroxylase inhibitor, was noninferior to epoetin alfa in treating anemia associated with kidney disease. Erythropoietin signals through two receptors — the high-affinity homodimeric erythropoietin receptor (which is responsible for erythropoiesis) and the low-affinity heterodimeric beta common receptor–erythropoietin receptor. We have found that the beta common receptor–erythropoietin receptor directly interacts with and activates vascular endothelial growth factor receptor 2,[1] which could mediate angiogenesis, inflammation,[2] and perhaps atherosclerosis.[3] The mechanism of activation of the beta common receptor–erythropoietin receptor

may explain the cardiovascular adverse effects of erythropoietin; a post hoc analysis has shown that the erythropoietin dose is an important determinant of adverse outcomes.[4] The use of prolyl hydroxylase inhibitors results in erythropoietin levels that are well in excess of 50 mIU per milliliter, the dissociation constant for the beta common receptor–erythropoietin receptor.

Although an oral medication offers some advantages, we caution that the long-term safety and efficacy of these agents remain unproved. Although erythropoietin was approved for use in 1989, the side effects of erythropoietin therapy were not recognized for approximately 30 years. Long-term clinical trials with adequate power are essential to evaluate the safety and efficacy of prolyl hydroxylase inhibitors.

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TO THE EDITOR: In the editorial that accompanied the articles by Chen et al. on two pivotal trials of roxadustat, an oral HIF prolyl hydroxylase inhibitor, Kaplan[1] discusses the increased incidence of hyperkalemia in the roxadustat groups. The editorialist stated, "hyperkalemia has not been reported as an adverse event in phase 2 trials of other HIF prolyl hydroxylase inhibitors, so hyperkalemia may be an off-target effect of roxadustat rather than a class effect." This may not be an accurate assessment, since phase 2 trials of other HIF prolyl hydroxylase inhibitors have revealed hyperkalemia. For example, Pergola et al.[2] studied vadadustat (another HIF prolyl hydroxylase inhibitor) and found hyperkalemia in 7 of 138 patients who received vadadustat, as compared with none of the 72 patients who received placebo. In a study of daprodustat by Meadowcroft et al.,[3] hyperkalemia was an adverse event in 8 of 177 patients who received daprodustat, as compared with none of the 39 controls. Although larger phase 3 trials are under way to provide a more complete picture, it appears that hyperkalemia may be a class effect of HIF prolyl hydroxylase inhibitors. These observations should help to guide further trial design and clinical monitoring.

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Dr. Fishbane reports receiving research support and consulting fees from AstraZeneca. No other potential conflict of interest relevant to this letter was reported.

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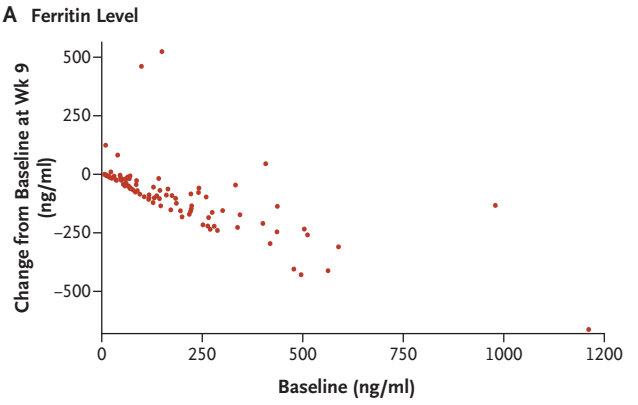
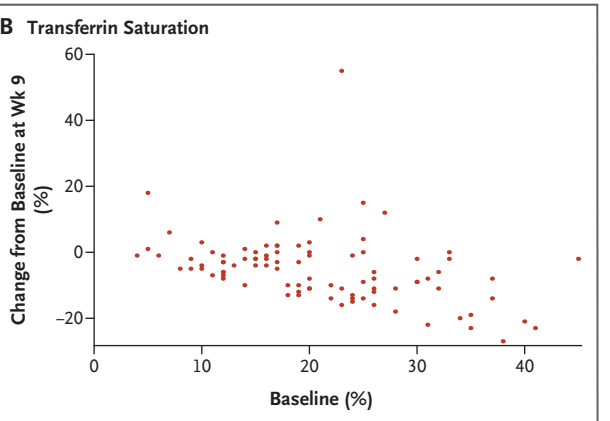
THE AUTHOR AND A COLLEAGUE REPLY: Anker et al. offer a hypothesis regarding the proportion of patients who received roxadustat and in whom iron deficiency developed in the two phase 3 trials showing the safety and efficacy of roxadustat in treating anemia in patients with chronic kidney disease. The temporary decrease in the transferrin saturation and ferritin level in patients who received roxadustat indicated that iron was necessary for erythropoiesis. In the Chinese FGCL-4592-808 trial, the largest temporary decreases in the ferritin level and transferrin saturation occurred in patients with the highest baseline values, and the decreases in these values were smallest in patients with lower iron stores (Fig. 1A and 1B). These findings were similar to those in a U.S. phase 2 trial of roxadustat in patients who were not undergoing dialysis.[1] Furthermore, in that 24-week U.S. trial, the transferrin saturation in the patients with a low baseline transferrin saturation recovered to the baseline level by week 20 and further increased to above





C Hyperkalemia in Three Trials

Reference	Trial	Dialysis	Treatment		No. of Participants		Average Duration of Treatment		Hyperkalemia	
			Intervention	Active Control	Roxadustat Group	Control Group	Roxadustat Group	Control Group	Roxadustat Group	Control Group
									%	
Provenzano et al.[2]	HIMALAYAS	Incident dialysis	Roxadustat	Epoetin alfa	522	521	1.5 yr	1.5 yr	5.0	7.0
Charytan et al.[3]	SIERRAS	Stable dialysis	Roxadustat	Epoetin alfa	370	371	1.7 yr	2.1 yr	16.2	15.1
Coyne et al.[4]	ANDES	None	Roxadustat	Placebo	616	306	95.6 wk	52.1 wk	11.5	10.5

Figure 1. Ferritin Levels, Transferrin Saturation, and Hyperkalemia in Recent Trials.

Panel A shows a scatterplot of the ferritin level at baseline as compared with the change in the ferritin level from baseline to week 9 in the FGCL-4592-808 trial of roxadustat in patients with chronic kidney disease who were not undergoing dialysis. Panel B shows a scatterplot of the transferrin saturation at baseline as compared with the change in transferrin saturation from baseline to week 9 in that trial. Panel C shows the rates of hyperkalemia in the roxadustat (intervention) and control groups in three recent global phase 3 trials.[2-4]

the baseline level by week 24 with roxadustat. These findings provide support for iron mobilization as part of coordinated erythropoiesis with roxadustat as well as for the new iron repletion standard for treating anemia with roxadustat rather than erythropoiesis-stimulating agents in patients with chronic kidney disease. Anker et al. highlight the potential effect of roxadustat on potassium metabolism. In the FGCL-4592-808 and FGCL-4592-806 trials, hyperkalemia occurred more often in the roxadustat group than in the comparator groups. However, analyses of central laboratory data did not show any clinically significant changes in potassium levels over time or between the groups. In the protocols of the two trials, the criteria for reporting hyperkalemia as an adverse event were not specified. Thus, evaluation of the objective central laboratory data on potassium levels are critical, considering the subjective judgments in adverse-event reporting.

Zielniok et al. comment on the potassium levels and metabolic acidosis in patients in the two trials in light of cell-culture data based on vadadustat, a different HIF prolyl hydroxylase inhibitor. They report that vadadustat up-regulated expression of key glycolytic genes in human bone marrow cells and indicate that increased glycolysis could lead to tissue acidification and hyperkalemia. We have concerns about generalization of the results from one HIF prolyl hydroxylase inhibitor to another, and it is important to recognize that results of cell-culture studies may not translate to clinically relevant effects.

Finally, we agree with Mohandas and Segal that the long-term safety and efficacy results of roxadustat will be critically important. Global phase 3 trials have been undertaken to provide further data on roxadustat. Preliminary results of trials that were recently reported at the American Society of Nephrology Kidney Week confirm the cardiovascular safety of roxadustat, and the incidences of hyperkalemia in the roxadustat groups and the epoetin alfa or placebo groups in these large global trials are similar[2-4] (Fig. 1C).

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THE EDITORIALIST REPLIES: Shah and Fishbane write, "hyperkalemia may be a class effect of HIF prolyl hydroxylase inhibitors." Data from trials of five different agents in the HIF prolyl hydroxylase inhibitor class are available. Roxadustat was associated with an increased risk of hyperkalemia. Vadadustat was associated with an increased risk of hyperkalemia in the phase 2 trial cited by Shah and Fishbane, although subsequent trials of vadadustat did not show an increased risk of hyperkalemia.[1,2] In the trial cited by Shah and Fishbane, the incidence of clinically important hyperkalemia was greater in the control group than in the daprodustat group; this finding calls into question the meaning of the reported adverse events of hyperkalemia. In patients who were not undergoing dialysis, daprodustat was not associated with an increased risk of hyperkalemia.[3] Trials of desidustat and molidustat did not show an increased risk of hyperkalemia.[4,5] A class effect should be associated with all members of a class, and thus hyperkalemia does not appear to be a class effect of HIF prolyl hydroxylase inhibitors.

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Since publication of his editorial, the author reports no further potential conflict of interest.

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