Case Series

Four Cases of Serum Copper Excess in Patients with Renal Anemia Receiving a Hypoxia-Inducible Factor-Prolyl Hydroxylase Inhibitor: A Possible Safety Concern

Hironori Nakamura   Shigekazu Kurihara   Mariko Anayama
Yasushi Makino   Masaki Nagasawa

Department of Nephrology, Shinonoi General Hospital, Nagano, Japan

Abstract

Copper is an indispensable trace metal element and is mainly absorbed in the stomach and small intestine and excreted into the bile. Hypoxia-inducible factor-prolyl hydroxylase inhibitors (HIF-PHIs) have emerged as a novel approach for renal anemia management. Many intestinal genes, including *divalent metal transporter 1*, *duodenal cytochrome B*, and copper transporter ATPase7A, related to iron absorption are transactivated by HIF-α, during iron deficiency. We first report 4 cases of patients with renal anemia who showed excess in serum copper level during roxadustat or daprodustat treatment, which were decreased to the normal level after discontinuing HIF-PHIs and changing the drug to darbepoetin alfa, suggesting that HIF-PHI is associated with serum copper excess. HIF-PHI modulates iron metabolism, such as iron absorption, sequestration, and mobilization, and may increase serum copper levels by increasing copper absorption and/or redistribution of copper in tissues. Therefore, it is urgent to examine the correlation between HIF-PHI use and serum copper levels because copper excess might be involved in several acute or chronic adverse events.

© 2022 The Author(s).
Published by S. Karger AG, Basel

Correspondence to:
Hironori Nakamura, hnakamur0525@gmail.com

Karger

Introduction

Copper is an indispensable trace metal element and is mainly absorbed in the stomach and small intestine and excreted into the bile [1]. Excess amounts of copper are toxic, and dysregulation causes severe complications, such as Menkes disease or Wilson disease [2].

Hypoxia-inducible factor (HIF)-prolyl hydroxylase inhibitors (HIF-PHIs) have emerged as a novel approach for renal anemia management. Many intestinal genes, including *divalent metal transporter 1 (DMT1)*, *duodenal cytochrome B (DCYTB)* [3], and copper transporter ATPase7A (ATP7A) [4], related to iron absorption are transactivated by HlF-α. Some studies have shown that DMT1 can transport copper [5, 6], while iron and copper have similar physiochemical properties and points of interaction [7]. Although HIF-PHIs have been well tolerated in clinical trials, there are still potential specific concerns [8]. Here, we first report 4 cases of patients with renal anemia who showed excess in serum copper level during HIF-PHI treatment, which were normalized after discontinuing HIF-PHI and changing to darbepoetin alfa, suggesting that HIF-PHI may be associated with serum copper excess.

Case Reports

Case 1

A 79-year-old male patient on hemodialysis was prescribed 70 mg of roxadustat three times a week because 120 μg/week of darbepoetin alfa was unable to maintain the target hemoglobin level. He had a complication of pancytopenia. Recent studies have shown vitamin B1 deficiency with normal bone marrow findings. The copper level (normal range 60–130 μg/dL) was 61.8 μg/dL during darbepoetin alfa treatment. After changing to roxadustat, the hemoglobin level was increased to approximately the target range; however, elevated serum copper levels were observed at 47, 56, and 70 days after the start of roxadustat (shown in Table 1; Fig. 1). The patient manifested appetite loss and nausea; therefore, roxadustat was changed to darbepoetin alfa. The decreased copper levels were observed at 13 and 65 days after the change. After the discontinuation of roxadustat, his appetite loss gradually improved. The zinc levels were below the normal range at every evaluation point (Table 1). The patient had approximately 500 mL/day of urine.

Case 2

A 67-year-old male with autosomal dominant polycystic kidney disease on peritoneal dialysis was prescribed 70 mg of roxadustat three times a week. A high copper level was observed at 6 months after roxadustat initiation. Roxadustat was changed to 4 mg of daily daprodustat because of the possible relationship between increased copper levels and roxadustat. Although a slightly decreased serum copper level was observed 28 days after the change, the copper level was still above the upper limit. Thus, daprodustat was changed to darbepoetin alfa, resulting in normal copper level at 22 days after the change (shown in Table 1; Fig. 1). The patient had approximately 1,000 mL/day of urine.

Case 3

An 80-year-old male patient with nondialysis-dependent end-stage renal disease had been treated with darbepoetin alfa for 3 years. Darbepoetin alfa was changed to 4 mg of daily daprodustat to achieve better anemia control 1 year ago. Laboratory findings showed elevated copper levels at 354 and 382 days after the start of daprodustat treatment. Daprodustat was changed to darbepoetin alfa because of the possible contribution to the serum copper level. Consequently, the copper level decreased to the normal range after 35 days of the change

Nakamura et al.: Four Cases of Serum Copper Excess

Table 1. Days and laboratory data including hemoglobin level, albumin, aspartate aminotransferase, alanine aminotransferase, iron-related parameters, and zinc level before, during, and after the HIF-PHI treatment. Halftone dot meshing shows the term of HIF-PHI treatment and its dose

Case 1								
Days before the start of roxadustat	186							
Days after the start of roxadustat		4	28	47	56	70		
Days after the discontinuation of roxadustat							13	65
Hemoglobin (11.6–14.8 g/dL)	9.7	8.5	10.3	9.2	9.7	9.0	6.7	7.0
Albumin (4.1–5.1 mg/dL)	3.2	3.3	3.3	n.a.	2.9	2.8	2.5	n.a.
Aspartate aminotransferase (13–30 U/L)	9	17	14	n.a.	20	34	**36**	n.a.
Alanine aminotransferase (10–42 U/L)	10	18	9	n.a.	15	29	37	n.a.
Copper (66–130 µg/dL)	61	n.a.	n.a.	**148**	**175**	**160**	100	62
Zinc (80–160 µg/dL)	44	n.a.	n.a.	45.8	n.a.	n.a.	27	32.3
Iron (40–188 µg/dL)	137	135	**232**	n.a.	**285**	**300**	**240**	126
Ferritin (20–280 ng/mL)	126	118	126	n.a.	n.a.	286	**370**	211
Transferrin saturation, %	73	69	96	n.a.	96	97	96	68
Roxadustat, mg		100	100	120	70	70	70	
Case 2								
Days before the start of roxadustat	0							
Days after the start of roxadustat		27						
Days after the start of daprodustat			124	152	180			
Days after the discontinuation of daprodustat						22		
Hemoglobin (11.6–14.8 g/dL)	10.7	11.8	11.9	11.2	12.0	11.3		
Albumin (4.1–5.1 mg/dL)	3.7	3.7	3.7	3.1	3.4	3.5		
Aspartate aminotransferase (13–30 U/L)	9	11	17	11	14	18		

Table 1 (continued)

Alanine aminotransferase (10–42 U/L)	7	8	12	7	8	15	13
Copper (66–130 µg/dL)	n.a.	n.a.	n.a.	n.a.	**164**	**133**	109
Zinc (80–160 µg/dL)	n.a.	n.a.	n.a.	n.a.	57.2	50.1	53.2
Iron (40–188 µg/dL)	80	66	139	58	105	94	99
Ferritin (20–280 ng/mL)	92	54	135	189	131	191	212
Transferrin saturation, %	31	20	46	24	35	34	40
Roxadustat or daprodustat, mg		100	100	100	100	4	
Case 3							
Days before the start of daprodustat	140						
Days after the start of daprodustat		91	165	256	354	382	
Days after the discontinuation of daprodustat							35
Hemoglobin (11.6–14.8 g/dL)	8.8	9.7	10.7	12.2	12.4	12.6	11.3
Albumin (4.1–5.1 mg/dL)	3.4	3.3	3.1	3.0	3.0	3.1	3.2
Aspartate aminotransferase (13–30 U/L)	20	23	30	20	22	17	27
Alanine aminotransferase (10–42 U/L)	9	7	9	7	6	8	15
Copper (66–130 µg/dL)	n.a.	n.a.	n.a.	n.a.	**155**	**159**	106
Zinc (80–160 µg/dL)	n.a.	n.a.	n.a.	n.a.	n.a.	62	61
Iron (40–188 µg/dL)	72	111	53	148	n.a.	88	126
Ferritin (20–280 ng/mL)	207	130	244	137	n.a.	143	338
Transferrin saturation, %	34	42	21	54	n.a.	32	64
Daprodustat, mg	4	4	4	4	4	4	

**Case Reports
in Nephrology
and Dialysis**

128

Case Rep Nephrol Dial 2022;12:124–131

DOI: 10.1159/000525735 | © 2022 The Author(s). Published by S. Karger AG, Basel
www.karger.com/cnd

Nakamura et al.: Four Cases of Serum Copper Excess

Table 1 (continued)

	Case 4		
Days before the start of daprodustat	119	1	
Days after the start of daprodustat			34
Days after the discontinuation of daprodustat				28
Hemoglobin (11.6–14.8 g/dL)	9.2	10.2	10.9	10.1
Albumin (4.1–5.1 mg/dL)	1.9	3.0	3.1	3.1
Aspartate aminotransferase (13–30 U/L)	16	16	14	17
Alanine aminotransferase (10–42 U/L)	9	19	10	14
Copper (66–130 µg/dL)	n.a.	n.a.	**154**	114
Zinc (80–160 µg/dL)	n.a.	n.a.	55	55.3
Iron (40–188 µg/dL)	43	65	90	50
Ferritin (20–280 ng/mL)	245	187	114	88.7
Transferrin saturation, %	19	34	38	23
Daprodustat, mg			2	

Bold indicates above normal range. n.a., not available.

Case Reports
in Nephrology
and Dialysis

Case Rep Nephrol Dial 2022;12:124–131

| DOI: 10.1159/000525735 | © 2022 The Author(s). Published by S. Karger AG, Basel www.karger.com/cnd |

Nakamura et al.: Four Cases of Serum Copper Excess
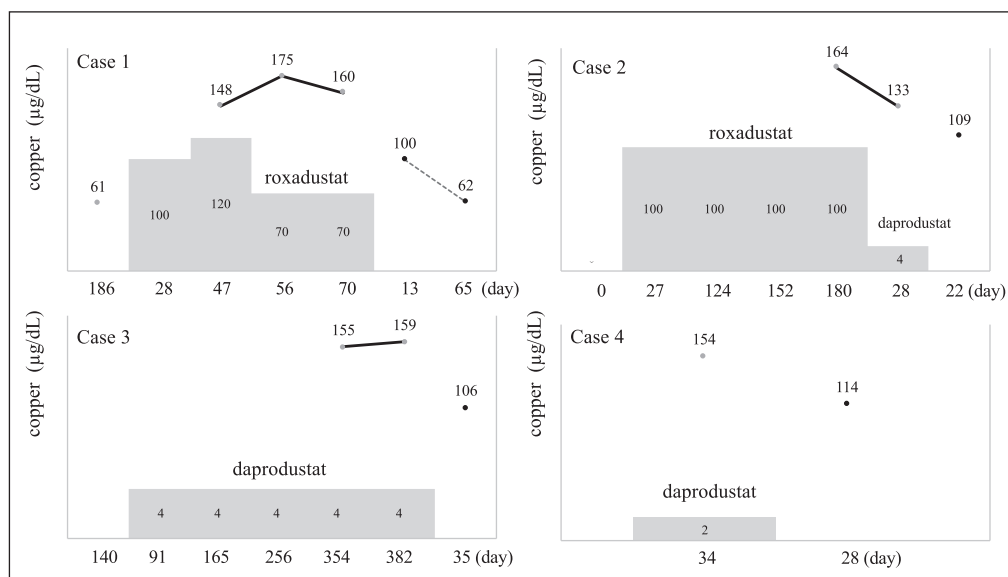
129

Fig. 1. Changes of serum copper days before, during, and after the HIF-PHI treatment. Halftone dot meshing shows the term of HIF-PHI treatment and its dose.

(shown in Table 1; Fig. 1). The patient had 7–10 mL/min/1.73 m^2 of estimated glomerular filtration ratio.

Case 4

A 66-year-old male patient on hemodialysis for 8 years was prescribed 2 mg of daily daprodustat. Laboratory data showed higher copper level at 34 days after initiation of daprodustat. Thus, daprodustat was changed to darbepoetin alfa. The copper level was normalized at 28 days after the change (shown in Table 1; Fig. 1). The zinc levels were below the normal range at every evaluation point. The patient had anuria.

In all cases, serum copper concentration was measured using the Quick Auto Neo Cu (SHINO-TEST Co., Sagamihara, Japan). Briefly, as described in the package insert, serum copper ions bound to protein (ceruloplasmin) are released using a deproteinizing agent. The copper ion reduced using ascorbic acid is reacted with a predetermined chelating agent, 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-(3-sulfopropyl) aniline derivative, and the copper concentration in the specimen is evaluated by measuring the change in color intensity of the sample.

Discussion

HIF-PHI was administered to 8 patients, and 4 of them showed copper excess as presented. Although one of the 4 cases revealed that serum iron levels changed in a way similar to copper levels, the other cases did not. In all four present cases, serum zinc levels did not change during HIF-PHI treatment. Although the precise mechanisms are currently unknown, we can raise possibilities that elevation in serum copper level is closely related to HIF-PHI treatment. First, HIF-2α mediates the adaptive increase in iron absorption during both systemic iron deficiency and erythropoietic demand under systemic hypoxia through direct transcriptional activation of the iron absorptive machineries [9, 10]. Moreover, copper uptake was enhanced by DMT1 during iron deprivation [5, 6]. In a rat model, roxadustat significantly increased

expression of *DMT1* and *DCYTB* mRNA and stabilized HIF-1α and HIF-2α in vitro. HIF-α protein levels rapidly declined after washout of roxadustat from cell cultures [11]. Thus, it is possible that HIF-PHIs, roxadustat, and daprodustat could increase iron and copper absorption by DMT-1 in enterocytes, resulting in excess serum iron and copper levels. Although case 1 was the only case that showed excess level in both iron and copper, excess of both these metals was normalized after the discontinuation of roxadustat treatment. Second, ATP7A, an enterocyte copper exporter, is induced at transcriptional level by HIF-2α [4], and probably that it is involved in the export of copper ions to the bloodstream [7, 12]. Thus, copper is likely redistributed to tissues including enterocytes, the liver, and the blood. Thus, copper redistribution may be associated with serum copper excess during HIF-PHI treatment. HIF-PHI may modulate not only iron but also copper metabolism as iron and copper have similar physio-chemical properties and points of interaction [7]. Overload of copper mainly includes two aspects: acute copper toxicity, such as nausea and vomiting and chronic toxicity [13]. A meta-analysis demonstrated that the HIF-PHIs group showed increased risk of diarrhea, nausea, and peripheral edema compared with the placebo group [14]. Manifestations of nausea in case 1 might be associated with copper excess probably induced by roxadustat. This report exhibited incidental cases that showed copper excess during anemia treatment with HIF-PHI in clinical practice. Unfortunately, we have no data on the serum copper levels before HIF-PHI treatment, except for case 1, but these clinical courses and mechanisms through HIF activations suggest that HIF-PHI increases serum copper levels by increasing copper absorption and/or redistribution in tissues.

In conclusion, serum copper excess may occur during the treatment of HIF-PHI regardless of the agent type, dose, and treatment term. Therefore, it is urgent to examine the correlation between HIF-PHI and serum copper levels because copper excess might be involved in some acute or chronic adverse events.

Statement of Ethics

Written informed consent was obtained from all patients for publication of the details of their medical case and any accompanying images. Ethical approval is not required for this case report in accordance with the Ethics Review Board of the Shinonoi General Hospital.

Conflict of Interest Statement

All authors declare that they have no relevant financial interests.

Funding Sources

Author Contributions

Research idea, study design, data acquisition, and data analysis/interpretation: Hironori Nakamura; supervision or mentorship: Shigekazu Kurihara, Mariko Anayama, Yasushi Makino, and Masaki Nagasawa. Hironori Nakamura takes responsibility that this study has been reported honestly, accurately, and transparently and accepts 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.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Wang P, Yuan Y, Xu K, Zhong H, Yang Y, Jin S, et al. Biological applications of copper-containing materials. Bioact Mater. 2021 Oct;6(4):916–27.

2 Mercer JF. The molecular basis of copper-transport diseases. Trends Mol Med. 2001;7(2):64–9.

3 Mastrogiannaki M, Matak P, Keith B, Simon MC, Vaulont S, Peyssonnaux C. HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. J Clin Invest. 2009 May;119(5):1159–66.

4 Xie L, Collins JF. Transcriptional regulation of the Menkes copper ATPase (Atp7a) gene by hypoxia-inducible factor (HIF2{alpha}) in intestinal epithelial cells. Am J Physiol Cell Physiol. 2011 Jun;300(6):C1298–305.

5 Wang X, Flores SR, Ha JH, Doguer C, Woloshun RR, Xiang P, et al. Intestinal DMT1 is essential for optimal assimilation of dietary copper in male and female mice with iron-deficiency anemia. J Nutr. 2018 Aug;148(8):1244–52.

6 Jiang L, Garrick MD, Garrick LM, Zhao L, Collins JF. Divalent metal transporter 1 (Dmt1) mediates copper transport in the duodenum of iron-deficient rats and when overexpressed in iron-deprived HEK-293 cells. J Nutr. 2013 Dec;143(12):1927–33.

7 Doguer C, Ha JH, Collins JF. Intersection of iron and copper metabolism in the mammalian intestine and liver. Compr Physiol. 2018 Sep;8(4):1433–61.

8 Yap DYH, McMahon LP, Hao CM, Hu N, Okada H, Suzuki Y, et al, -PHI Recommendation Committee. Recommendations by the Asian Pacific society of nephrology (APSN) on the appropriate use of HIF-PH inhibitors. Nephrology. 2021 Feb;26(2):105–18.

9 Anderson ER, Xue X, Shah YM. Intestinal hypoxia-inducible factor-2alpha (HIF-2alpha) is critical for efficient erythropoiesis. J Biol Chem. 2011 Jun;286(22):19533–40.

10 Taylor M, Qu A, Anderson ER, Matsubara T, Martin A, Gonzalez F, et al. Hypoxia-inducible factor-2α mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. Gastroenterology. 2011 Jun; 140(7):2044–55.

11 Del Balzo U, Signore PE, Walkinshaw G, Seeley TW, Brenner MC, Wang Q, et al. Nonclinical characterization of the hypoxia-inducible factor prolyl hydroxylase inhibitor roxadustat, a novel treatment of anemia of chronic kidney disease. J Pharmacol Exp Ther. 2020 Aug;374(2):342–53.

12 Jończy A, Mazgaj R, Starzyński RR, Poznański P, Szudzik M, Smuda E, et al. Relationship between down-regulation of copper-related genes and decreased ferroportin protein level in the duodenum of iron-deficient piglets. Nutrients. 2020 Dec;13(1):104.

13 Franchitto N, Gandia-Mailly P, Georges B, Galinier A, Telmon N, Ducassé JL, et al. Acute copper sulphate poisoning: a case report and literature review. Resuscitation. 2008 Jul;78(1):92–6.

14 Chen H, Cheng Q, Wang J, Zhao X, Zhu S. Long-term efficacy and safety of hypoxia-inducible factor prolyl hydroxylase inhibitors in anaemia of chronic kidney disease: a meta-analysis including 13,146 patients. J Clin Pharm Ther. 2021 Aug;46(4):999–1009.