Successful application of roxadustat in the treatment of patients with anti-erythropoietin antibody-mediated renal anaemia: a case report and literature review

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Abstract
Recombinant human erythropoietin (rHuEPO) has been used worldwide for treatment of renal anaemia due to its good curative effect. However, rHuEPO treatment is associated with a rare but severe complication because of the development of anti-EPO antibodies, which are difficult to treat. Currently, the main treatments for the anti-EPO antibodies include withdrawing the rHuEPO, providing blood transfusions and administrating steroid-based immunosuppressive agents. Although the above methods can alleviate anti-EPO-related anaemia, there are obvious side-effects such as decreased immunity and an increased risk of infection. Therefore, accurately identifying anti-EPO-related anaemia and effectively treating this complication is worth exploring. This current case report describes a 49-year-old female patient with chronic kidney disease that received rHuEPO subcutaneously and then developed anti-EPO antibody-mediated renal anaemia with her haemoglobin levels dropping to 37 g/l. The patient refused to be treated with steroids, so she received 120 mg roxadustat administered orally every 72 h and her Hb level increased to 110 g/l over a few months. This current case report demonstrates that roxadustat can be used to successfully treat anti-EPO antibody-mediated renal anaemia without the use of steroid-based immunosuppressants.

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Keywords
Anti-erythropoietin antibodies, haemoglobin, roxadustat, hypoxia-inducible factor prolyl hydroxylase inhibitor

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Introduction
Recombinant human erythropoietin (rHuEPO) has been used throughout the world in the therapy of renal anaemia due to its good curative effect. However, rHuEPO has many side-effects, such as elevating blood pressure, forming thromboses and inducing anti-EPO antibodies. Although anti-EPO antibody production is rare, it is a serious complication during renal anaemia treatment, a condition that has limited therapeutic options. When anti-EPO antibodies occur, rHuEPO should be completely discontinued and transfusion is required to maintain the haemoglobin (Hb) level. Immunosuppressive agents are also used to inhibit the production of anti-EPO antibodies.

Previous literature has reported that roxadustat, an inhibitor of hypoxia-inducible factor proline hydroxylases (HIF-PHI), has effects on anti-EPO antibodies. As a novel oral agent, roxadustat first became available in China on 18th December 2018 and it has now been successfully used in treating patients with renal anaemia. Recently, roxadustat was found to be useful in treating anti-EPO antibody-mediated renal anaemia.

This current case report describes a female patient with chronic kidney disease that developed anti-EPO antibody-mediated renal anaemia subsequent to rHuEPO treatment, which was successfully treated with roxadustat.

Case report
A 49-year-old female patient with a 10-year history of glomerulonephritis underwent peritoneal dialysis in November 2013 at the Department of Nephrology, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, Zhejiang Province, China. In addition to continuous ambulatory peritoneal dialysis, the patient received 10 000 international units (IU) of rHuEPO (epoetin-z; Epogen®, 3SBio, Shenyang, China) administered subcutaneously every week from March 2014 to April 2016; and 150 mg iron polysaccharide complex capsules administered orally every day from November 2013 up to the present time (Kremers Urban Pharmaceuticals, Seymour, IN, USA) to maintain Hb levels at 90–113 g/l. However, in October 2016, this patient reported the major complaint of fatigue and her Hb level dropped sharply to 67 g/l. There was no bleeding, other acute haemorrhagic events or infections. Subsequently, a bone marrow biopsy was undertaken and there was no significant abnormality in the bone marrow. The result of an anti-EPO antibody test using an enzyme-linked immunosorbent assay kit (3SBio) was negative. According to her medical history and an examination of the results described above, the dose of rHuEPO was increased to a maximum dose of 6000 IU twice a week by subcutaneous injection. However, her Hb level did not increase as expected and declined to 37 g/l, which required a blood transfusion to maintain her Hb level >60 g/l. In
March 2018, another bone marrow biopsy was undertaken and the result was similar to that in 2016. Based on the test results, rHuEPO was discontinued and she received intermittent transfusion therapy because her Hb level was 40–60 g/l.

To identify the cause of her anaemia, the patient was admitted again to the Department of Nephrology, HwaMei Hospital on 7 December 2019. After admission, the patient received a comprehensive evaluation including laboratory tests. The complete blood counts were as follows: white blood cell count $8.2 \times 10^9$/l; Hb 45 g/l; haematocrit 13.1%; platelet count $331 \times 10^9$/l; and reticulocyte count $7 \times 10^9$/l. The levels of the components of the blood, such as iron, vitamin B12 and folic acid, were sufficient. Based on the existing medical history and examinations, multiple myeloma, systemic autoimmune disease, gastrointestinal bleeding and parvovirus B19 infection were excluded. However, she needed repeated blood transfusions due to the low Hb levels. Meanwhile, another anti-EPO antibody test was undertaken to determine the reason for her declining Hb levels and it was positive. The patient was given the diagnosis of anti-EPO antibody-mediated renal anaemia.

With regard to her subsequent treatment, rHuEPO was discontinued and steroids were not administered because the patient refused to take them. The patient received 120 mg roxadustat (FibroGen, San Francisco, CA, USA) administered orally every 72 h from 10 January 2020. After 2 months of treatment, her Hb level had reached 90 g/l and her symptoms of fatigue had significantly improved without side-effects. The last follow-up indicated that her Hb level was 110 g/l (Figure 1).

The research protocols conformed to the provisions of the Declaration of Helsinki and were approved by the Ethics Committee of University of Chinese Academy of Sciences, Ningbo, Zhejiang Province, China (no. PJ-NBEY-KY-2020-183-01). The patient provided written informed consent for publication of their data.

**Discussion**

This current case report describes the successful use of the HIF-PHI roxadustat for the treatment of a female patient with anti-EPO antibody-mediated renal anaemia without the need for the use of steroid-based immunosuppressants.

The occurrence of anti-EPO antibodies, first identified in 1996, is the primary reason for EPO resistance at 3–67 months after use. The incidence of anti-EPO antibodies was reported to be 1.27/1000 (95% confidence interval 0.3 to 3.7/1000) patient-years since the start of dialysis. In vitro research has shown that these antibodies may inhibit the binding of EPO to its receptor, blocking the differentiation of erythroid progenitors. In addition, anti-EPO antibodies may cause pure red cell aplasia (PRCA), which is a disease characterized by an absence of red blood precursors in the bone marrow and reticulocytopenia. It has also been reported that in PRCA, there were normal platelet and neutrophil production levels with positive anti-EPO antibodies after rHuEPO treatment. In this current case, PRCA was excluded due to the normal results from the first two bone marrow biopsies. However, one patient was diagnosed with PRCA among three patients who had anti-EPO antibodies in the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD) study. Therefore, the current patient was suspected of being in the early stage of PRCA despite there being no significant changes in her bone marrow tests.

With regard to the cause of anti-EPO antibodies in this current case, it might have been associated with the rHuEPO itself, treatment modality or drug storage.
rHuEPO-α and rHuEPO-β are produced in Chinese hamster ovary cells using recombinant methods. The two forms of rHuEPO have slight differences in glycosylation; rHuEPO-α has more sialic acid residues than rHuEPO-β. When the patient’s immune function is active, anti-EPO antibodies may be produced against heterogeneous glycosyl groups. A previous study identified 13 patients that had anti-EPO antibodies during treatment with rHuEPO and suggested that the epitope targeted by the anti-EPO antibodies was the protein rather than the carbohydrate moiety. The underlying mechanisms of the association between the anti-EPO antibodies and PRCA require further research. The second factor that might affect the immunogenicity of rHuEPO is the treatment modality. Previous studies have shown that the incidence of PRCA following the subcutaneous injection of rHuEPO was higher than that after administration via an intravenous injection. The EPO-related incidence of PRCA was 1.7/10 000 in France, while the rate was 0.26/10 000 in Germany, and the preference for using subcutaneous injections of rHuEPO in France might explain this difference. Delivering the rHuEPO via a subcutaneous injection exposes the drug to more antigen-presenting cells and allows for a more prolonged duration for protein absorption. The third factor that might affect the immunogenicity of rHuEPO is the how it is stored and the use of an EPO stabilizer. rHuEPO is susceptible to increased temperature during storage and transport. Any denaturation caused during a failure of the cold chain may be the key inducer of antibody production, which was thought to be related to the EPO stabilizer polysorbate-80 (PS-80). Research has shown that PS-80 can increase protein aggregation and drug immunogenicity. Also, the exposure of hidden epitopes of rHuEPO may increase the immunogenicity under altered conditions of packaging, storage and transport. In clinical practice, healthcare professionals...

Figure 1. Changes in haemoglobin (Hb) and haematocrit (HCT) levels during the clinical course in a 49-year-old female patient with a 10-year history of glomerulonephritis that underwent peritoneal dialysis and treatment with recombinant human erythropoietin (rHuEPO). The colour version of this figure is available at: http://imr.sagepub.com. qw, once a week; ih, subcutaneous; biw, twice a week; q5d, once every 5 days; q72h, once every 72 h.
should be advised to store rHuEPO at 2–8°C; and avoid interrupting the cold chain or exposing the drug to freezing conditions, shock or light.

The main methods for testing for anti-EPO antibodies are enzyme-linked immunosorbent assays and radioimmuno-precipitation assays. However, their specificity and sensitivity are not high. In this current case, the first test for anti-EPO antibodies was negative, followed by a second, later test that was positive. It was not clear whether there were anti-EPO antibodies at the first test. Moreover, in the NECOSAD study, one patient was found to have anti-EPO antibodies at their third and fourth tests and another patient was not diagnosed as having anti-EPO antibodies until their fifth test. Therefore, it is suggested that several repeated anti-EPO antibody tests are conducted to exclude the possibility of false-negative results when EPO resistance appears to be a likely cause of renal anaemia.

The treatment of anti-EPO antibody-mediated renal anaemia usually involves the withdrawal of rHuEPO and the initiation of immunosuppressive therapy. After this treatment strategy, the anti-EPO antibodies disappear in 78% of all cases with reticulocyte counts returning to 10 000/ml. However, the current patient refused to take steroids. Furthermore, a continuous EPO receptor stimulator, effective in managing PRCA, was not available in China. Roxadustat, as an oral HIF-PHI, can stimulate endogenous EPO in the physiological range and increase Hb concentrations. Due to its efficacy and safety in treating renal anaemia, 120 mg roxadustat administered orally every 72 h was used to treat this patient without any steroid-based immunosuppressive therapy. The Hb level increased from 45 g/l to 110 g/l over a few months, indicating the successful treatment of anti-EPO antibody-mediated renal anaemia using roxadustat alone. Roxadustat, a drug with an entirely different chemical structure to that of rHuEPO, can avoid stimulating the subcutaneous immune system and bring benefits to patients that have anti-EPO antibodies. Furthermore, recent research has demonstrated the other roles of roxadustat, which include suppression of humoral immunity, cell proliferation and inhibition of the differentiation of CD4+ T cells into type 1 helper T and type 17 helper T cell subsets. These other actions might explain why this current case achieved the desired outcome without using steroid-based immunosuppressive agents. This current case was different to another patient that received roxadustat after using immunosuppressant agents.

In conclusion, careful evaluation of this current case suggests that repeat testing for anti-EPO antibodies is required when a patient is suspected of having developed resistance to EPO therapy. Roxadustat was useful in treating anti-EPO antibody-mediated renal anaemia without the use of immunosuppressants in this current case.

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Declaration of conflicting interest
The authors declare that there are no conflicts of interest.

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References

1. Chapter 3: Use of ESAs and other agents to treat anemia in CKD. Kidney Int Suppl (2011) 2012; 2: 299–310.
2. Watson AJ. Adverse effects of therapy for the correction of anemia in hemodialysis patients. Semin Nephrol 1989; 9: 30–34.
3. Casadevall N, Natf J, Viron B, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med 2002; 346: 469–475.
4. Rossert J, Casadevall N and Eckardt KU. Anti-erythropoietin antibodies and pure red cell aplasia. J Am Soc Nephrol 2004; 15: 398–406.
5. Verhelst D, Rossert J, Casadevall N, et al. Treatment of erythropoietin-induced pure red cell aplasia: a retrospective study. Lancet 2004; 363: 1768–1771.
6. Wu Y, Cai X, Ni J, et al. Resolution of epoetin-induced pure red cell aplasia, successful re-challenge with roxadustat. Int J Lab Hematol 2020; 42: e291–e293.
7. Chen N, Hao C, Liu BC, et al. Roxadustat Treatment for Anemia in Patients Undergoing Long-Term Dialysis. N Engl J Med 2019; 381: 1011–1022.
8. Chen N, Hao C, Peng X, et al. Roxadustat for Anemia in Patients with Kidney Disease Not Receiving Dialysis. N Engl J Med 2019; 381: 1001–1010.
9. Casadevall N, Dupuy E, Molho-Sabatier P, et al. Autoantibodies against erythropoietin in a patient with pure red-cell aplasia. N Engl J Med 1996; 334: 630–633.
10. Kharagjitsingh AV, Korevaar JC, Vandenbroucke JP, et al. Incidence of recombinant erythropoietin (EPO) hyporesponsiveness, EPO-associated antibodies, and pure red cell aplasia in dialysis patients. Kidney Int 2005; 68: 1215–1222.
11. Krantz SB. Pure red cell aplasia. N Engl J Med 1974; 291: 345–350.
12. Storring PL, Tiplady RJ, Gaines Das RE et al. Epoetin alfa and beta differ in their erythropoietin isoform compositions and biological properties. Br J Haematol 1998; 100: 79–89.
13. Macdougall IC, Roger SD, de Francisco A, et al. Antibody-mediated pure red cell aplasia in chronic kidney disease patients receiving erythropoiesis-stimulating agents: new insights. Kidney Int 2012; 81: 727–732.
14. Brinks V, Hawe A, Basmeleh AH, et al. Quality of original and biosimilar epoetin products. Pharm Res 2011; 28: 386–393.
15. Locatelli F, Del Vecchio L and Pozzoni P. Pure red-cell aplasia “epidemic”—mystery completely revealed? Perit Dial Int 2007; 27(Suppl 2): S303–S307.
16. Schellekens H. Bioequivalence and the immunogenicity of biopharmaceuticals. Nat Rev Drug Discov 2002; 1: 457–462.
17. Weber G, Gross J, Kromminga A, et al. Allergic skin and systemic reactions in a patient with pure red cell aplasia and antierythropoietin antibodies challenged with different epoetins. J Am Soc Nephrol 2002; 13: 2381–2383.
18. Wu G, Wadgymar A, Wong G, et al. A cross-sectional immunosurveillance study of anti-EPO antibody levels in CRF patients receiving epoetin alfa in 5 Ontario Renal Centers. Am J Kidney Dis 2004; 44: 264–269.
19. Urra JM, de la Torre M, Alcazar R, et al. Rapid method for detection of anti-recombinant human erythropoietin antibodies as a new form of erythropoietin resistance. Clin Chem 1997; 43: 848–849.
20. Lim SK, Bee PC, Keng TC, et al. Resolution of epoetin-induced pure red cell aplasia 2 years later, successful re-challenge with continuous erythropoiesis receptor stimulator. Clin Nephrol 2013; 80: 227–230.
21. Besarab A, Provenzano R, Hertel J, et al. Randomized placebo-controlled dose-ranging and pharmacodynamics study of roxadustat (FG-4592) to treat anemia in nondialysis-dependent chronic kidney disease (NDD-CKD) patients. Nephrol Dial Transplant 2015; 30: 1665–1673.
22. Eleftheriadis T, Pissas G, Mavropoulos A, et al. In Mixed Lymphocyte Reaction, the Hypoxia-Inducible Factor Prolyl-Hydroxylase Inhibitor Roxadustat Suppresses Cellular and Humoral Alloimmunity. Arch Immunol Ther Exp (Warsz) 2020; 68: 31.