Epoietin-induced Antibody-mediated Pure Red Cell Aplasia and Responses to Immunosuppression Therapy: 2 Case Reports and Literature Review

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Abstract

Recombinant human erythropoietin (rHuEPO) induced antibody-mediated pure red cell aplasia (PRCA) was a rare disease. Herein, we reported 2 cases with confirmed diagnosis of EPO- induced antibody-mediated PRCA in our institution. Case 1: a 53 year-old female with uremia and regular hemodialysis was regularly administered with EPO-α (Eprex) subcutaneously. Progressive unexplained anemia was noted 13 months after therapy with Eprex. Bone marrow study revealed remarkable erythroid hypoplasia compatible with PRCA. Anti-EPO antibody levels were detected. After withdrawal of Eprex and administration of cyclosporine, her anemia gradually improved. Serum level of anti-EPO antibody became undetectable. Another form of EPO (darbepoetin) was administered to the patient and hemoglobin recovered to 10.4 g/dL. Case 2: a 46 year-old male with chronic kidney disease related anemia underwent a regular subcutaneous EPO-β (Recormon) therapy. He developed profound anemia in spite of dose increase of EPO-β and combination with darbepoetin usage. EPO induced antibody-mediated PRCA was confirmed by the detection of anti-EPO antibody and severe erythroid hypoplasia in the bone marrow. Cessation of EPO and initiation of immunosuppression therapy with methylprednisolone and azathioprine were instituted. The patient finally became transfusion-independent. (J Intern Med Taiwan 2010; 21: 441-447)

Key words: Pure red cell aplasia, Anti-erythropoietin antibodies, Immunosuppression therapy, Dialysis, Erythropoietin, Uremia

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Introduction

Recombinant erythropoietin (EPO) is used successfully to treat anemia in chronic kidney disease (CKD), to maintain stable hemoglobin levels, and to avoid the need for multiple transfusions. Recently, antibody-mediated PRCA has been associated with the administration of EPO and identified as a serious complication. The incidence of epoetin-associated PRCA increased considerably in 1998, reached a peak in 2002, and decreased sharply since 2003[1]. EPO discontinuation and administration of immunosuppressive treatment can accelerate recovery from PRCA induced by EPO[2]. Here, we report 2 cases of EPO associated PRCA, the successful management of their diseases, and a review of related literature.

Case Report

Case 1

A 53 year-old female had a history of hypertension and chronic glomerulonephropathy. She developed uremia with regular hemodialysis since December 2002. Eprex 2,000U (Johnson & Johnson; uncoated rubber stopper, polysorbate 80 as stabilizer) was administered subcutaneously biweekly for her chronic kidney disease (CKD) associated anemia. She suffered from mild itchiness after Eprex injection. Exacerbation of anemia began in January 2004, which was 13 months after initiation of Eprex therapy. Hematological data showed a hematocrit 22.1%. A full examination for blood loss (including stool occult blood, upper and lower gastrointestinal endoscopies) was negative. There was no evidence of hemolysis or hemoglobinopathies or iron, vitamin B12, or folate deficiencies. No typical symptoms and signs of parvovirus B19 infection were noted. A low reticulocyte count of 3,013/μL was also observed. A radioimmunoprecipitation (RIP) test detected the presence of anti-EPO antibodies. Bone marrow aspiration smear revealed a scarcity of erythroid precursors compatible with PRCA (Fig.1). Cyclosporine (2~3mg/kg/day initially from June 2004 to May 2005) and prednisolone (25mg/day) was instituted. Anti-EPO antibody level was rechecked and it became undetectable by 8 months post treatment. To treat her anemia, darbepoetin (Aranesp) 40μg was prescribed weekly since April 2005. A good response was observed at 7 months later and darbepoetin dose was reduced to 25μg/week. The hemoglobin gradually increased to 10.4 g/dL. The patient had recovered from rHuEPO-induced PRCA and successfully switched to another erythropoiesis-stimulating agent.

Case 2

A 46 year-old male had CKD and was started on EPO-β (Recormon, Roche) 2,000U s.c. biweekly in February 2006 to maintain hemoglobin at 8~10 g/dL (Fig.2). Disease progression to uremia was noticed, and he underwent peritoneal dialysis. However, his hemoglobin gradually dropped to 6~8g/dL in spite of regularly administered Recormon. Recormon dose was increased to 5,000u biweekly s.c., but his anemia continued to deteriorate. His hemoglobin declined to 4.0 g/dL and he became transfusion-dependent (PRBC 4units/month). Aranesp was added 25 μg once

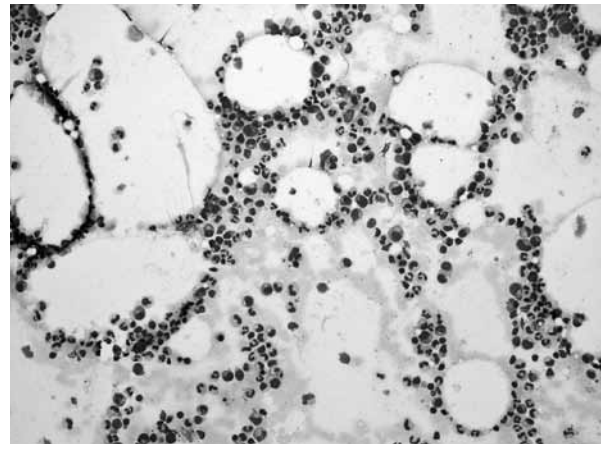

Fig. 1. Case 1. Severe erythroid hypolasia and severe scarcity of erythroid precursor. Myeloid cells/erythroid cells (M/E) ratio was about 30:1 (bone marrow aspiration, ×400, Liu stain).

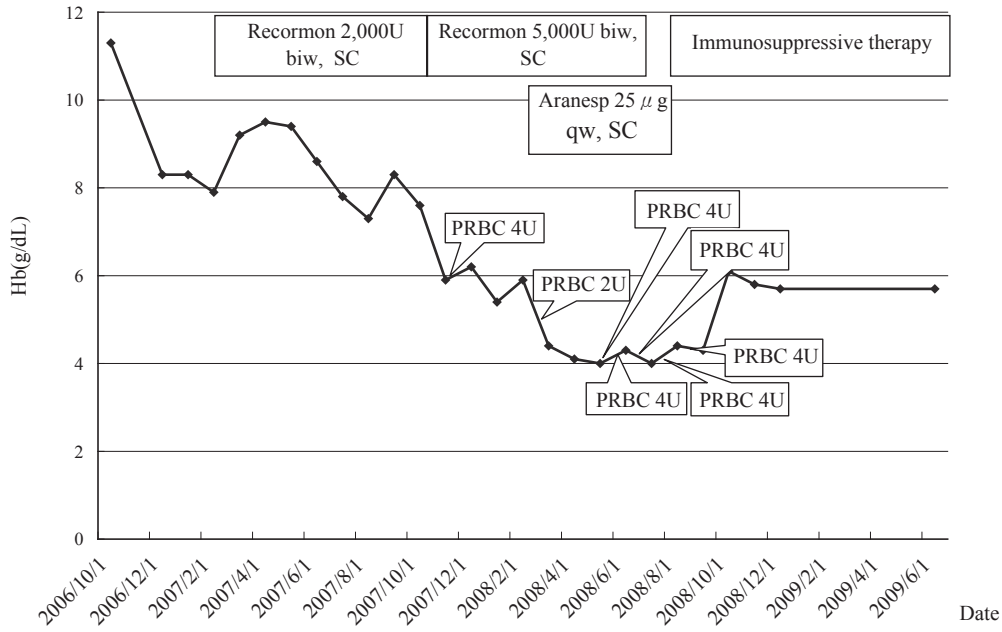

Fig. 2. Clinical course of epoietin treatment, blood transfusion, Hb level and immunosuppression therapy in case 2.

weekly, but in vain. Further workup of his anemia revealed negative occult blood test of stool. Upper and lower gastrointestinal endoscopies also showed negative finding. Ferritin level was 552ng/mL. No evidence showed parvovirus B19 infection. Investigations to ascertain a cause for the anemia were essentially negative. Therefore, he was referred to our hematology clinic. Lab data revealed Hb 5.4 g/dL, Hct 15.2%, MCV 82.8fL, reticulocyte 1,190/μL. Bone marrow examination showed a marked decrease in erythroid series, with a less than 5% of total nucleated cells which was compatible with PRCA by 8 months post rHuEPO (Fig.3). Serum anti-EPO antibody levels were measured by an enzyme-linked immunosorbent assay (ELISA) kit, MicroCoat Biotechnologie GmbH. The confirmatory assay demonstrated detectable anti-EPO antibody level 212ng/ml. We started immunosuppressive therapy with methylprednisolone at 8mg daily and azathioprine at 50mg daily since Aug 2008, and discontinued rHuEPO. His hemoglobin gradually increased to 5.8g/dL, and blood transfusions were not required. However this patient lost to follow-up afterwards.

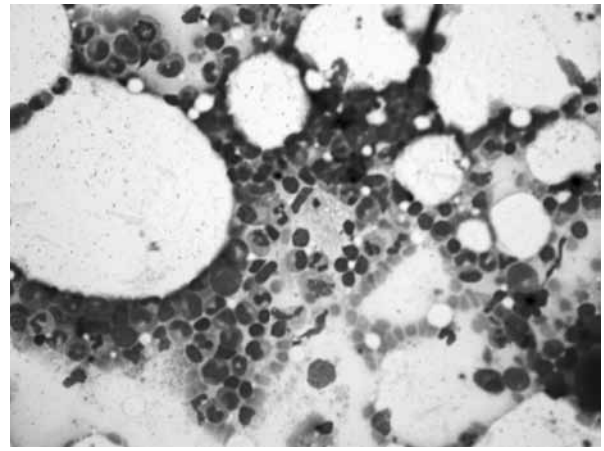

Fig. 3. Case 2. Marked erythroid hypoplasia. Erythroid series were less than 5% of total nucleated cells. M/E ratio more than 15:1 (bone marrow aspiration, ×1000, Liu stain).

Discussion

EPO induced PRCA is a rare condition. Patients may develop antibody against the EPO protein. The antibody neutralizes all currently available erythropoiesis-stimulating agents (ESA) and the patient's endogenous EPO. The proposed diagnostic criteria published by an ad hoc international working group of expert hematologists and nephrogist (table 1)[3] included progressive

Table 1. Clinical features of epoetin-induced pure red cell aplasia (PRCA)[3]

Major features (each of the major criteria should be identified in all cases)
- Treatment with epoetin for at least 3 weeks
- Drop of hemoglobin of about 0.1 g/dL per day without transfusions or transfusion need of about 1 unit per week to keep hemoglobin level stable
- Very low reticulocyte count (usually below 10 ×109/L)
- Usually normal white cell and platelet counts

Minor features
- Skin and systemic allergic reactions

Confirmational investigations
- Bone marrow aspirate shows virtual absence of red cell precursors (<5% erythroblasts) with evidence of a maturation block in the presence of normal cellularity
- Serum assay shows presence of anti-erythropoietin antibodies and evidence of their neutralizing capacity

anemia, low absolute reticulocyte count, severe scarcity of erythroblasts in bone marrow, resistance to ESA therapy, and presence of anti-EPO neutralizing antibody. Both of our cases met these criteria.

EPO antibodies are identified by a variety of assays, including RIP assays, ELISA, and surface plasmon resonance procedures.[2] Neutralizing activity of the antibodies was confirmed by an in vitro bioassay[4]. Bioassays are relatively time-consuming and only a minority of cases reported the results. We used RIP assays in case 1 and ELISA test in case 2. The reason why we used different methods was because the samples were sent to referent laboratories.

Only 3 cases of EPO-associated PRCA were reported prior to 1998, but almost 200 cases were described in patients who had CKD and were treated with ESA injected subcutaneously between 1998 and 2004[5]. The possible mechanisms of EPO-PRCA were as follows: First, human serum albumin (HSA) was replaced with polysorbate 80 to avoid Creutzfeldt–Jakob disease (CJD)[6]. In 1998, the HSA in the formulation of Eprex marketed in Europe was replaced with polysorbate 80 as a stabilizer. Subsequently, a sharp increase in EPO-PRCA occurred. Most cases of PRCA outside the US with epoetin-α have been attributed to polysorbate 80 in place of HSA as a stabilizer for epoetin[7]. Second, the route of subcutaneous injection especially self-administered with storage and handling issues has the potential to augment antibody formation[8]. Third, increased incidence of PRCA was associated with leachates from the uncoated rubber stopper used in pre-filled syringes. The leachates increased EPO aggregation which enhanced its antigenicity. In mid-2003, the manufacturer of HSA-free Eprex replaced the rubber stopper of pre-filled syringes with Teflon-coated plungers. Subsequently, the exposure-adjusted incidence rate decreased 13-fold with intravenously administrated Eprex[7]. Silicon oil was a lubricant in the pre-filled syringe of Eprex and was another possible cause of increased immunogenicity[7]. Fourth, polysorbate 80 and polysorbate 20 (stabilizer of Recormon) may induce the formation of epoetin-containing micelles[9] and increase immunogenicity. The polysorbate 80 concentration (0.03%) in Eprex is higher than polysorbate 20 concentration (0.01%) in Recormon. The higher risk of Eprex in EPO-induced PRCA than Recormon appears related to higher concentration of micelles and their related immune reactions. Furthermore, Darbepoetin in Europe and Canada is formulated with lower polysorbate 80 concentration than the Eprex formulation. (0.005% vs 0.03%)[10]. Fifth, processing, such as freeze-drying, and

formulations that facilitate the oxidation or aggregation of protein can enhance immunogenicity[7]. Heat, sunlight and other stressful environmental conditions may cause denaturation of proteins, which may increase their potential immunogenicity[11]. Sixth, the glycosylation of EPO may reduce immunogenicity, and has been called the shielding effect. Carbohydrates on EPO molecules can also increase molecular stability, solubility, and in vivo biological activity[12]. While more glycosylation sites may decrease the immune reaction in some cases, the EPO analogue darbepoetin-α carries two additional glycosylation sites that permit a higher degree of glycosylation than Eprex and Recormon[13]. In case 1, the possible causes of EPO related PRCA might be the subcutaneous route of injection, uncoated rubber stopper, and the stabilizer of Eprex (polysorbate 80) related problems. In case 2, the subcutaneous injection route should be most responsible for EPO related PRCA.

National authorities and manufacturers of EPO are collaborating to resolve the induction of EPO-related PRCA. Intravenous (iv) administration appeared less likely than subcutaneous administration to evoke an immune response. In 2002, European Union regulatory authorities mandated that CKD patients should be administered with eprex i.v. instead of s.c. and the annual number of Eprex associated PRCA declined 90%[10]. In 2003 and 2004, regulatory authorities in Canada, Singapore, and Australia mandated hemodialysis patients to receive administration of Eprex i.v. and nondialyzed CKD patients to receive darbepoetin s.c. Consequently, only six cases of Eprex-related PRCA have been reported[9].

We propose that immediate cessation of particular erythropoiesis-stimulating agents and initiation of immunosuppressive agents are required to treat EPO related PRCA. However, an exception was seen in a patient who suffered from Eprex related PRCA and responded well to darbepoiectin with persistant EPO antibodies without immunosuppressive therapy.[14] The most commonly used immunosuppressive agents were steroids, cyclosporine, cyclophosphamide, and rituximab[15]. Mycophenolate mofetil, plasma exchange and immunoadsorption[16] were occasionally used with varying effectiveness. Kidney transplantation is an alternative approach in patients with EPO associated PRCA[17]. In a retrospective study enrolling 47 patients with EPO related PRCA indicated Eprex (Ortho biotech) administered subcutaneously was the most common culprit[18]. The median time between the start of EPO treatment and the occurrence of PRCA was 11 months. Nine patients received no immunosuppressive treatment, and none of these recovered. Of the 37 patients who received immunosuppressive therapy, 29 (78%) recovered. Six of six patients who received a kidney transplant completely recovered within 1 month. These patients were treated with induction therapy followed by triple immunosuppressive agents. No relapse of PRCA occurred in any patients who recovered, even though immunosuppressive therapy was stopped. Nevertheless, no recovered patients were subsequently treated with EPO[19]. In case 1, we used cyclosporine and the patient was recovered completely. In case 2, we tried azathioprine, but the effect was modest though the patient became transfusion-independent.

Conclusion

The incidence of EPO- induced PRCA has decreased in recent years after collaboration of national authorities and manufacturers. Nevertheless, physicians should be alert to EPO-induced PRCA, if CKD patients experience poor response to EPO, rapid decline of hemoglobin after exposure of ESA, and extremely low reticulocyte counts. Increased EPO dosage and initiation of another EPO should be avoided until the patient is evaluated for anemia and exclusion of EPO induced PRCA. Further investigations

including a bone marrow exam and EPO antibodies measurement should be undertaken. Patients, who developed EPO-related PRCA, may benefit from cessation of EPO and initiation of immunosuppression therapy. Kidney transplantation may cure the disease. Administration with a different ESA could be considered in those patients with undetectable EPO antibodies.

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紅血球生成素引起之抗體導致的純紅血球再生不良以及用免疫抑制療法有效的治療此疾病：二則病例報告以及文獻回顧

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摘 要

基因重組人類紅血球生成素所引起之純紅血球再生不良是一種罕見疾病，在此我們報告馬偕醫院確診的 2 個基因重組人類紅血球生成素所引起之純紅血球再生不良的病例。病例一爲一位五十三歲尿毒症並接受規則洗腎的女性，平時接受規律的紅血球生成素 α(Eprex) 皮下注射。在 Eprex 使用 13 個月後病人被發現有不明原因且逐漸惡化的貧血。骨髓抽吸檢查顯示紅血球系列再生不良，且符合純紅血球再生不良的診斷。抗紅血球生成素抗體亦被偵測出。在停用 Eprex 且使用環孢靈治療後，病人的貧血逐漸改善。且病人血液中亦偵測不到抗紅血球生成素抗體。之後給病人使用另一種類的紅血球生成素 (darbepoetin) 治療。病人最後貧血完全復原。病例二是一位 46 歲的男性，因慢性腎病引起的貧血接受規則的紅血球生成素 β(Recormon) 皮下注射治療。儘管使用更高劑量的 Recormon 以及 darbepoetin 治療，他的貧血卻越來越惡化。病人血液中可偵測到抗紅血球生成素抗體。因此我們停止使用紅血球生成素以及使用免疫抑制的藥物類固醇和 azathioprine 治療。病人貧血逐漸改善而不需要輸血。