CASE REPORT

Successful treatment of a hemodialyzed patient with pure red cell aplasia associated with epoetin beta pegol therapy with cyclosporine

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Abstract A 42-year-old man with end-stage renal failure had been receiving hemodialysis therapy since April 2009. Initially, darbepoetin alfa was administered to treat his renal anemia. After treatment was switched to epoetin beta pegol, the patient's hemoglobin levels rapidly decreased. He was diagnosed with pure red cell aplasia (PRCA) based on the results of a bone marrow examination. Epoetin beta pegol was strongly suspected to be the cause of the PRCA, and although he tested negative for anti-epoetin beta pegol antibodies, epoetin beta pegol was discontinued and cyclosporine therapy was initiated. Thereafter, his hemoglobin levels increased, and his anemia dramatically improved after 3 months.

Keywords Cyclosporine · Epoetin beta pegol · Hemodialysis · Pure red cell aplasia

Introduction

Erythropoietin-stimulating agents (ESA) are widely used for the treatment of renal anemia in patients with chronic kidney disease to maintain optimal hemoglobin levels.

These agents have been reported to be safe and effective for the management of renal anemia [1]. Epoetin beta pegol is a third-generation ESA, and its half-life is known to be about 20 times longer than that of erythropoietin itself [2]. Recently, an increase in the incidence of pure red cell aplasia (PRCA) associated with ESA therapy has been reported [3]. However, in Japan, reports of PRCA induced by ESA therapy have been scarce [4, 5], and PRCA associated with epoetin beta pegol has not yet been reported. Here we report a case of PRCA associated with epoetin beta pegol in a hemodialyzed patient. The patient's anemia dramatically improved as a consequence of the discontinuation of epoetin beta pegol and administration of cyclosporine.

Case report

The patient was a 42-year-old man who was receiving hemodialysis (HD) therapy since April 2009 for the treatment of end-stage renal failure due to chronic glomerulonephritis. The complete clinical course of the patient is depicted in Fig. 1.

The patient's hemoglobin levels were maintained around 9–10 g/dL through the administration of epoetin beta before the initiation of HD and darbepoetin alfa after HD initiation. In August 2012, darbepoetin alfa was switched to epoetin beta pegol to improve the patient's renal anemia. Although his hemoglobin levels temporarily increased after the switch to epoetin beta pegol treatment, they started to decrease 1 month later and within 3 months of the switch, they had decreased to 7.8 g/dL. The result of a fecal occult blood test was negative, and the serum levels of ferritin, folic acid, and vitamin B12 were all within their normal ranges. The reticulocyte count was relatively low

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Fig. 1 Clinical course of the patient. A bone marrow biopsy was performed in October 2013 (↑). The patient received red blood cell transfusion six times (↑). The patient's hemoglobin levels markedly improved after the discontinuation of epoetin beta pegol and the start of cyclosporine treatment. *CERA* epoetin beta pegol, *CyA* cyclosporine, *DA* darbepoetin alfa, *EPO* epoetin beta, *RBC* red blood cell, *Hb* hemoglobin, *IU* international unit, *W* week, and *D* day

(13,260/ μ L) compared with the degree of anemia; therefore, the decrease in hemoglobin level may have been due to a diminished erythropoietin response.

In November 2012, epoetin beta pegol was switched back to darbepoetin alfa. However, this change proved ineffective at managing the renal anemia. Thus, the patient was treated with epoetin beta again, and his renal anemia improved. Thereafter, his hemoglobin levels were maintained at around 8–9 g/dL by administering epoetin beta at a dose of 9000 IU/week. However, to increase his hemoglobin levels to the target range for the management of renal anemia in HD patients [6], epoetin beta pegol treatment replaced epoetin beta in August 2013. After this switch, the patient's hemoglobin levels rapidly decreased to 4.8 g/dL within several weeks, and this decrease was not accompanied by an increase in reticulocyte counts. Further examination, including an endoscopy of the upper gastrointestinal tract and a fecal occult blood test, could not detect any cause for the decrease in hemoglobin level. Hence, a bone marrow biopsy was performed. The result of this examination showed the complete absence of erythroid precursors with normal myeloid and lymphoid subsets. Based on this finding, the patient was diagnosed with PRCA. A detailed description of the laboratory data of the patient at the time of the PRCA diagnosis is shown in Table 1.

Cytogenetic analysis showed a normal male karyotype. The level of anti-epoetin beta pegol antibody was examined in October 2013 in Chugai Pharmaceutical Co. Ltd Kamakura Research Laboratories using a bridging enzyme-

linked immunosorbent assay and radioimmunoprecipitation assay, which used the patient's serum itself as a primary antibody [7]. However, both of these tests showed negative results. Results of serological tests for parvovirus immunoglobulin M (IgM), cytomegalovirus IgM, Epstein Barr virus IgM, and antinuclear antibody were also negative. Serum complement concentrations, such as C3 and C4, were within the normal range. A computed tomography scan of the chest and abdomen ruled out the possibility of thymoma, lymphoma, or other tumors. His medications included cinacalcet 25 mg/day, omeprazole 10 mg/day, febuxostat 10 mg/day, sevelamer 750 mg/day, and calcium carbonate 1500 mg/day, which had not been changed since January 2012.

Based on these results, the cause of PRCA was strongly suspected to be epoetin beta pegol administration even though the patient tested negative for anti-epoetin beta pegol antibodies. Epoetin beta pegol was discontinued, and treatment was switched again to epoetin beta because epoetin beta had been effective on the worsening anemia caused by the initial administration of epoetin beta pegol. In addition, cyclosporine administration was initiated at a dose of 100 mg/day for 2 weeks and 150 mg/day thereafter. Cyclosporine was given in a divided dosage schedule twice a day, and the cyclosporine trough level was measured just before the dose was taken. The trough level was 154 ng/mL at a dose of 150 mg/day. After 4 weeks, increases in the reticulocyte counts and hemoglobin levels were observed, and thereafter the patient's hemoglobin levels dramatically recovered to levels higher than 10 g/dL

Table 1 Laboratory data at the time of the pure red cell aplasia diagnosis

Examination	Value	Normal range
White blood cells (/μL)	4810	3900–9800
Band cell (%)	2.0	0–19
Segmented cell (%)	54.0	25–72
Lymphocyte (%)	11.0	19–48
Monocyte (%)	13.0	3.4–9.0
Eosinophil (%)	17.0	0–7.0
Basophil (%)	3.0	0–2.0
Red blood cells (/μL)	182×10^4	$427\text{--}570 \times 10^4$
Hemoglobin (g/dL)	6.3	12–17.6
Hematocrit (%)	19.2	39.8–51.8
Mean corpuscular volume	105.5	83–101
Reticulocytes (%)	0.1	0.4–3.0
Platelets ($\times 10^3/\mu\text{L}$)	520	130–369
Ferritin (ng/mL)	434	25–280
Serum iron (μg/dL)	229	70–130
Total iron binding capacity (μg/dL)	247	187–405
Vitamin B12 (pg/mL)	346	233–914
Folate (ng/mL)	2.8	3.6–12.9
Lactate dehydrogenase (IU/L)	161	110–220
Aluminum (μg/dL)	≤ 10	≤ 16
Haptoglobin (mg/dL)	185	41–273
Direct coombs	Negative	
Parvovirus IgM	Negative	
Parvovirus IgG	Negative	
Cytomegalovirus IgM	Negative	
Cytomegalovirus IgG	Negative	
Cytomegalovirus antigen	Negative	
Epstein Barr virus IgM	Negative	
Epstein Barr virus IgG	Positive	
Epstein Barr virus PCR	$<2.0 \times 10$	
Human immunodeficiency virus IgM	Negative	
Human immunodeficiency virus IgG	Negative	
Antinuclear antibody	40	≤ 40
Anti-epoetin beta pegol antibody (ELISA)	0.0039	≤ 0.0062
Anti-epoetin beta pegol antibody (RIPA)	62	≤ 433

ELISA enzyme-linked immunosorbent assay, *IgG* immunoglobulin G, *IgM* immunoglobulin M, *RIPA* radioimmunoprecipitation assay

in January 2014; however, red blood cell transfusions, totaling 12 units, were necessary to maintain his hemoglobin levels before the increase in the reticulocyte and hemoglobin levels in his clinical course.

Discussion

PRCA is a disorder characterized by normocytic anemia associated with reticulocytopenia and an absence of erythroblasts. The acquired form of PRCA has been reported

to be caused by parvovirus infection, collagen disease, leukemia, lymphoma, thymoma, or treatment with ESAs or other drugs (such as phenytoin, azathioprine, and isoniazid) [8]. In our case, the PRCA diagnosis was made based on the Japanese PRCA guideline [9]. Other possible causes for the PRCA, including a lymphoproliferative disorder, thymoma, viral infection, an autoimmune condition, or drugs were excluded. Considering that both patient's hemoglobin levels and reticulocyte counts gradually decreased during the first period of epoetin beta pegol administration and sharply dropped during the second period, we strongly

suspect that epoetin beta pegol treatment led to the development of PRCA although there was no anti-epoetin beta pegol antibody. Regarding the relationship between ESA-associated PRCA and the presence or absence of anti-ESA antibodies, cases of PRCA induced by ESA therapy that did not show anti-ESA antibodies have previously been reported [10, 11]. The pathogenesis of ESA-associated PRCA without anti-ESA antibodies is largely unknown; however, it might be possible to associate this with the induction of autoimmunity [12]. Particularly in our case, the administration of epoetin beta pegol itself would lead to the development of PRCA via the increase in autoimmune activity associated with a pegylated glycoprotein. Epoetin beta pegol is a continuous erythropoietin receptor activator with a long half-life. Structurally, a pegylated glycoprotein consisting of a linear methoxy polyethylene glycol (PEG) molecule is attached to an amino acid residue of epoetin beta [13]. Pegylation is the process of covalent attachment of PEG polymer chains to another molecule, usually a drug or therapeutic protein. Pegylation markedly enhances the *in vivo* potency of recombinant human non-glycosylated erythropoietin and results in a longer half-life when compared to the non-pegylated form [14]. Although all ESAs contain various versions of glycoproteins, only epoetin beta pegol contains a pegylated glycoprotein. The pegylation method is also widely used for other drugs, such as granulocyte-colony stimulating factor, factor VIII, and interferon alfa [15]. Thus far, PRCA caused by pegylated interferon has previously been reported [16–18]. PEG is generally considered non-immunogenic by itself; however, a strong anti-PEG immune response was reported for some pegylated proteins, particularly ovalbumin [19], and mice exposed to adenovirus modified with a high molecular weight PEG showed a strong immune response compared to those exposed to the unmodified adenovirus [20]. Therefore, in our case, the induction of autoimmunity to the pegylated glycoprotein is the most probable pathogenesis of PRCA development including the inhibition of erythropoiesis, although we cannot exclude other possibilities because PRCA itself is an incidental complication in HD patients [11].

Regarding the treatment of PRCA associated with ESA therapy, it has previously been reported that PRCA did not improve without immunosuppressive treatment [21]. The largest retrospective study, including 47 patients with PRCA, reported that renal transplantation, corticosteroids, cyclosporine, and corticosteroids in combination with cyclophosphamide were considered effective therapies [22]. In our case, as the patient had a history of osteoarthritis of the bilateral hip joints, we thought that steroid administration would induce osteonecrosis at these

joints. Therefore, in our patient, cyclosporine was selected as a PRCA treatment.

In general, administration of a high induction dose of cyclosporine (5–8 mg/kg/day) is usually required for the treatment of PRCA. Thereafter, treatment with cyclosporine at a trough level of at least 150 ng/mL is necessary for maintaining remission [23]. In our case, the patient was taking omeprazole at the start of cyclosporine, and it is reported that omeprazole reduces the hepatic clearance of cyclosporine by inhibition of cytochrome P450 enzyme system, thus leading to higher cyclosporine levels with the same dosage [24]. Furthermore, the clearance rate of cyclosporine has shown to be diminished under uremic condition [25]. Therefore, we started low dosage of cyclosporine and increased the dosage gradually. As a result, a relatively low dose of cyclosporine (150 mg/day: 2.2 mg/kg/day) was sufficient to keep its trough level at 150 ng/mL and to successfully achieve a remission of PRCA associated with epoetin beta pegol. Additionally, the effectiveness of the low-dose cyclosporine administration in this HD case might be explained by the decrease in cyclosporine clearance in the liver.

In conclusion, we describe the case of a hemodialyzed patient with PRCA that was likely caused by epoetin beta pegol administration. The patient was successfully treated by cyclosporine administration and epoetin beta pegol discontinuation, and fully recovered after several months.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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