short report
Haematologica 1996; 81:143-147

RECOMBINANT HUMAN ERYTHROPOIETIN FOR LONG-TERM TREATMENT OF ANEMIA IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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ABSTRACT

The long-term effects of recombinant human erythropoietin (rhEPO) administration in two consecutive cases of paroxysmal nocturnal hemoglobinuria (PNH) with severe anemia are reported. In both patients, a 68-year-old woman and a 66-year-old man, a diagnosis of PNH was made on the basis of severe macrocytic anemia associated with hemoglobinuria, hemosiderinuria and positivity for the sucrose and Ham tests. Subcutaneous treatment with rhEPO, 150 U/Kg body weight daily, was followed in both cases by a progressive increase in hemoglobin concentrations, which thereafter were maintained above 10 g/dL with lower doses of rhEPO and without any relevant side effects for 32 and 29 months of continuous treatment, respectively. A clinical response was observed in spite of elevated baseline serum erythropoietin concentrations, appropriate to the degree of anemia in both patients. These results suggest that rhEPO may be appropriately and safely used in the long-term correction of anemia associated with PNH, and that the response to the pharmacologic doses of rHEPO administered was not dependent on the level of endogenous erythropoietin.

Key words: recombinant human erythropoietin; paroxysmal nocturnal hemoglobinuria; anemia; erythropoiesis

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of hematopoiesis due to a somatic mutation occurring at the stem cell level.[1]

Although PNH is an ancient disease, its crucial physiopathological mechanism has been shown only recently to arise from a somatic mutation in the pig-A gene, which leads to an acquired failure to express phosphatidylinositol glycan-anchored protein (PIG-AP) on the surface of blood cells, in particular erythrocytes, inducing in them an abnormal susceptibility to autologous complement activation.[2] The pig-A gene mutation is at the origin of the expansion of one or more progenitors, so that PNH is aptly defined as an acquired clonal disorder of hematopoiesis, and the circulating erythrocytes represent chimeric subpopulations of affected and normal cells.[3]

Clinically, PNH is characterized by either chronic or acute intravascular hemolysis, pancytopenia, increased susceptibility to infections and deep venous thrombosis, and it has been frequently associated with aplastic anemia as well as myelodysplastic syndromes or myelogenous leukemia.

Although several different therapeutical approaches have been proposed to correct PNH, so far there is no specific treatment for this disorder (with the possible exception of allogenic bone marrow transplantation in eligible candicates) and PNH patients are basically supported by blood transfusions and iron replacement.[4]

Given the effectiveness of recombinant human erythropoietin (rhEPO) in treating the anemia associated with chronic renal failure, the use of this hormone has been proposed in recent years for the treatment of several different forms of chronic anemia.[5]

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Acknowledgments: the authors wish to thank Dr. Njdia Testa for her critical reading of the manuscript.
Note: Some of the authors participate in trials supported by the Janssen Cilag pharmaceutical company, which provided rHuEpo for this study.
Received November 7, 1995; accepted January 31, 1996.

Good clinical response to high-dose, short-term rhEPO has been described by anectodal reports in a few patients with PNH,[6,7] but whether lower doses of rhEPO given as long-term treatment could be safe and effective in patients with PNH remains to be assessed.

Here we report the results of long-term treatment with rhEPO in two consecutive patients with severe anemia associated with PNH.

Case #1

A 68-year-old woman previously in good health started to experience exertional dyspnea in February 1991. In December 1991 fatigue suddenly worsened and she first reported macroscopic emission of *brownish* urine. The patient was referred to another hospital, where a diagnosis of PNH was made on the basis of mild macrocytic anemia (Hb 10.1 g/dL; MCV 114 fL), hemoglobinuria, hemosiderinuria and a positive acidified-serum lysis test (Ham's test).

The patient was discharged with no treatment except iron and folate supplementation, but anemia progressively worsened and she finally was admitted to our hospital in June 1992. At physical examination the patient appeared pale, but was otherwise normal.

The main clinical characteristics of the patient at the time of first admission are reported in Table 1. She presented macrocytic anemia (Hb 8.9 g/dL, MCV 95 fL) associated with reticulocytosis and marked signs of hemolysis (LDH 5680 U/L; indirect bilirubin 25.65 μm/L, haptoglobin 4 g/L). Hemoglobinuria and hemosiderinuria were also evident. Both the sucrose and Ham test were positive. Bone marrow examination showed increased cellularity with erythroid hyperplasia. Erythropoietin (EPO) serum levels were increased (187 U/L; normal range for our laboratory 5-16 U/L) and were appropriate for the degree of anemia.

Therapy with deflazacort (30 mg/daily) was begun and iron and folate supplementation were continued. The patient was discharged and followed on an outpatient basis, with her Hb levels ranging from 9.0 to 10.0 g/dL. In February 1993 she experienced a new episode of acute hemolysis and her Hb concentration fell to 7.3 g/dL. Two units of packed red cells (PRC) were given, deflazacort therapy was discontinued and subcutaneous (s.c.) treatment with rhEPO (EPREX, Janssen-Cilag)(150 U/kg body weight daily) was begun after informed consent. The course of Hb levels and reticulocyte count during rhEPO treatment are shown in Figure 1A. During the first few months of rhEPO treatment her Hb levels progressively increased to 12.4 g/dL, with noticeable improvement in her sense of well-being and without any side effects. RhEPO doses were subsequently adjusted in order to maintain Hb levels above 10.0 g/dL. Despite two episodes of frank hemolysis which required intravenous iron supplementation, she has not required any more transfusions, and a bone marrow examination performed after 24 months of continuous rhEPO treatment was almost unchanged with respect to the one obtained at first admission. At the time of this writing, after 31 months of continuous therapy with rhEPO, the patient is doing well, without any relevant side effects.

Table 1. Patient characteristics at the time of first admission.

Characteristics	*Case 1*	*Case 2*
Gender/age (years)	female/68	male/66
Duration of disease (months)	8	18
Hemoglobin (g/dL)	8.8	7.4
MCV (fL)	95	101
WBC (x 10^9/L)	5.3	1.4
Platelets (x 10^9/L)	345	150
Reticulocytes (x 10^9/L)	229	117
EPO* levels (U/L)	187	310
LDH (U/L)	5680	3670
Bilirubin (mmol/L)	25.65	29.0
Haptoglobin (g/L)	4	2
Bone marrow	erythroid hyperplasia	erythroid hyperplasia
Karyotype	46, xx	46, xy

**EPO = serum erythropoietin*

Case #2

A 66-year-old man was referred to our Institution in April 1993 for pancytopenia. The patient, previously in substantially good health, suddenly experienced fatigue associated for the first time with *brownish* urine in December 1991. A similar episode occurred one year later,

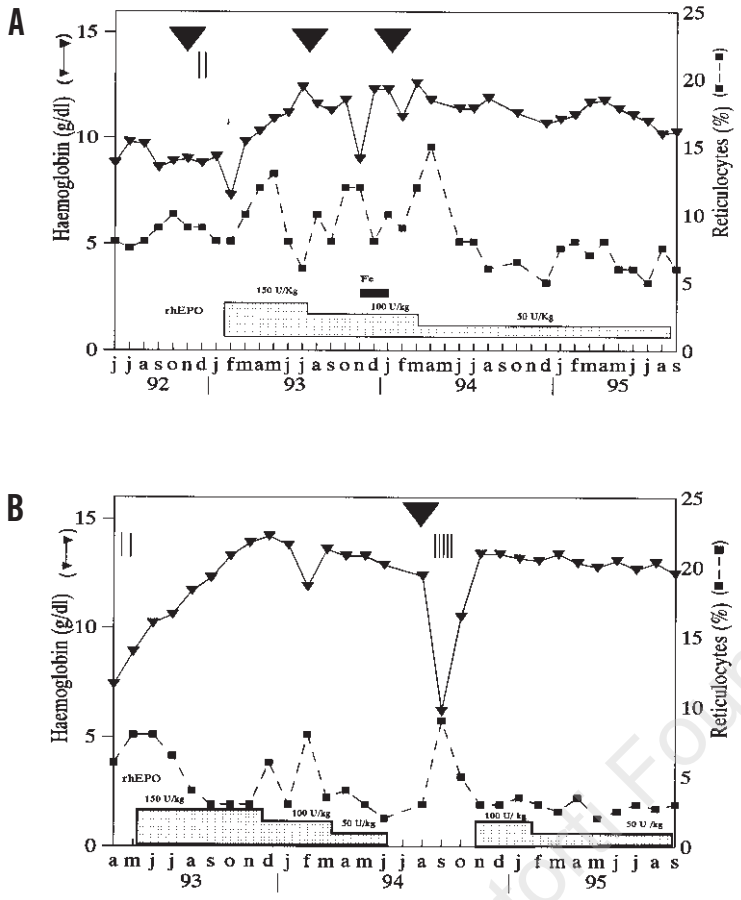

Figure 1. Effects of rhEPO treatment on hemoglobin (t— t) and reticulocytes (n- - -n). Arrow heads represent episodes of frank hemolysis. Solid bars indicate transfusion of one unit of packed red cells.

in December 1992, and then in March 1993 when the patient was finally admitted to another hospital, where macrocytic anemia (Hb 10.6 g/dL; MCV 99 fL), leukopenia (WBC 1.50 $\times 10^9$/L) and thrombocytopenia (107$\times 10^9$/L) were observed.

Clinical characteristics at the entry are reported in Table 1. A diagnosis of PNH was made on the basis of macrocytic anemia associated with signs of hemolysis, hemoglobinuria, hemosiderinuria, and positivity for both the sucrose and Ham tests. Bone marrow examination showed increased cellularity, with erythroid hyperplasia and mild signs of dyserythropoiesis. Chromosomal analysis revealed a normal karyotype. EPO serum levels were increased (310 U/L) and appropriate for the degree of anemia.

Treatment with rhEPO (150 U/kg body weight s.c. daily) was begun after informed consent and transfusion of two units of PRC in May 1993. The patient's Hb levels increased progressively during the following months to 14.2 g/dL in December 1993, as shown in Figure 1B.

In June 1994, rhEPO was stopped when the Hb level was near normal and only mild signs of microscopic hemoglobinuria were present. However, a new episode of marked hemolysis occurred in September 1994 after a short febrile illness, and transfusion of five units of PRC was required.

A bone marrow examination performed on that occasion showed no change. RhEPO was then restarted and the patient is continuing to do well, with no evidence of any side effects, 28 months after the first administration of the synthetic hormone.

Discussion

Different treatments have been proposed and utilized for the anemia of PNH, but of course real cures have been obtained only following allogeneic (or syngeneic) transplantation, that is after eradication of the faulty clone and engraftment of healthy stem cells.[4]

Since the first reports on the effectiveness of recombinant human erythropoietin for the treatment of the anemia associated with chronic renal failure, rhEPO administration has been proposed in several different forms of chronic anemia, including that associated with clonally pathological hematopoiesis such as in myelodysplastic syndromes and other hemopoietic stem cell disorders.[8]

Anecdotal responses to rhEPO in PNH have been reported recently.[6,7] In all these cases the hormone was used at very high doses and in short-term phase I or II clinical studies. In only one report was rhEPO utilized for 12 months in a patient with PNH, but at high doses and in combination with corticosteroids.[7]

In the two cases of severely anemic patients with PNH presented here, we observed a very good long-term clinical response to relatively low doses of rhEPO. Despite the fact that rather high (>100 U/L) baseline serum EPO concentrations were recently proposed as being predictive of poor response to rhEPO,[9] both our patients responded clinically even though they presented elevated baseline EPO concentrations that were substantially adequate for the degree of anemia. This finding is in agreement with other anecdotal responses to rhEPO observed in spite of appropriate endogenous erythropoietin production,[10] and it suggests that this criterion of predictability might not be applicable to PNH patients, who seem to respond to intermediate pharmacological doses of rhEPO.

The coexistence in PNH-affected individuals of normal hematopoiesis with the pathological PNH clone might account for this apparent discrepancy. Actually, whether it is the pathological clone or residual normal erythropoiesis that responds to rhEPO in this clinical disorder remains to be proved, although clinical responses by clonal pathological hematopoiesis to rhEPO have already been described.[11]

No special investigations were performed to assess this in our cases, but it appears logical to hypothesize that EPO-responsive cells reside in conserved unaffected clones, which may be more sensitive to the mitogenic[12] and anti-apoptotic effects of the hormone.

Furthermore, in addition to the absence of relevant side effects after 31 and 26 months, respectively, of rhEPO administration, bone marrow examinations failed to indicate any sign of myelodysplastic of leukemic transformation (both reported as possible evolutions of PNH), thus apparently indicating that this type of evolution is not facilitated by rhEPO.

In conclusion, our experience suggests that rhEPO may be appropriately used in the long-term correction of severe anemia associated with PNH, even though the cost of this treatment must be carefully considered with regard to appropriate utilization of financial resources. The savings in blood transfusions should also be taken into account.

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