Case Report

Two haemodialysis patients with epoetin alfa-induced pure red-cell aplasia recovered despite treatment with another epoetin preparation

Aarne Vartia¹, Markku R. Asola², Risto Tertti³, Pertti Kunelius² and Kaj P. Metsärinne³,⁴

¹Savonlinna Central Hospital, Savonlinna, ²Satakunta Central Hospital, Pori, ³Turku University Central Hospital, Turku and ⁴Helsinki University Central Hospital, Helsinki, Finland

Keywords: anaemia; antierythropoietin antibodies; darbepoetin; epoetin alfa; epoetin beta; pure red-cell aplasia

Introduction

In the past few years several cases of pure red-cell aplasia (PRCA) associated with neutralizing anti-erythropoietin antibodies emerging during epoetin treatment have been reported [1–6]. The majority of the cases have occurred in Europe and have been associated with subcutaneous administration of epoetin alfa [4,7,9,10]. There are very few reports from the USA. The epoetin alfa preparations used in USA are different from those used in Europe [7,10]. Discontinuation of epoetin therapy as well as immunosuppressive treatment have been used to treat the condition.

We report two patients who developed PRCA during subcutaneous epoetin alfa therapy. After discontinuation of epoetin alfa, both patients were successfully treated with other epoetins followed by a good response in erythropoiesis and without any signs of allergic reactions.

Cases

Case 1


One month after the initiation of dialysis his haemoglobin level was 92 g/l. Epoetin alfa was started 4000 U subcutaneously twice weekly. In April, haemoglobin was 94 g/l and the amount of hypochromic erythrocytes was 12.3%. Intravenous iron saccharate was given and during the following months, haemoglobin rose steadily reaching a level of 136 g/l at the end of year 2000. During the following 3 months, however, a rapid decline in haemoglobin values was seen, the lowest level being 76 g/l in March 2001. There were no signs of bleeding and blood reticulocyte count was clearly decreased to 9×10⁹/L. The patient received several repeated blood transfusions in addition to which the dose of epoetin alfa was gradually increased to 8000 U subcutaneously three times a week A bone marrow aspiration showed good iron stores, normal megacaryocytes and normal myelopoesis but almost no erythropoiesis. No signs of a viral infection, aplastic anaemia, or thymoma could be found. In April 2001, epoetin alfa was discontinued and epoetin beta was started 9000 U subcutaneously three times a week. From May 2001 on, the patient also received immunosuppressive therapy with cyclosporin, 75–125 mg twice daily. The haemoglobin values started to rise gradually. Repeated bone marrow aspirations were performed in July and September 2001, the former showing some improvement and the latter a normal erythropoiesis. By November 2001, haemoglobin had risen to 107 g/l and the blood reticulocyte count was 125×10⁹/L. Unfortunately, tests for antierythropoietin antibodies were not performed during the acute phase of the disease, but the test for neutralizing antibodies was inconclusive in January 2002, when the bone marrow had already recovered. By February 2002, haemoglobin had reached a level of 117 g/l and cyclosporin A was discontinued. Thereafter, the maintenance dose of epoetin beta has been 5000 U subcutaneously once a week.

Case 2

A 66-year-old female patient with type 2 diabetes since 1985 manifested diabetic nephropathy with proteinuria in 1999 (Figure 1 and Table 1).
Epoetin alfa 4000 U twice weekly subcutaneously was started in November 2000 at a haemoglobin level of 93 g/l. She responded as expected and haemoglobin level had reached 131 g/l in March 2001 when haemodialysis was started. In May, haemoglobin was 83 g/l. The patient received intravenous iron saccharate and epoetin dose was increased gradually to 10 000 U thrice weekly. In July, haemoglobin was 61 g/l, absolute reticulocyte count was 3 × 10^9/L and bone marrow aspiration showed PRCA. No thymoma was detected in computer tomography, there were no signs of a lymphoproliferative or autoimmune disease, and she did not have antibodies against parvovirus. Epoetin alfa was discontinued.

Four months later a trial of immunosuppressive therapy with cyclosporin 100–150 mg twice daily was started, from November 2001 to March 2002. In April 2002, bone marrow aspiration showed red cell aplasia as previously and reticulocyte count was still very low. Darbepoetin alfa 30 μg once weekly intravenously was started in April 2002. Immediate response was noted in erythropoiesis and, in May 2002, the patient received her last red cell transfusion. Altogether, she had received 55 U of red blood cells during the 12 months from June 2001. Since then, her haemoglobin level has been easily kept at tolerable levels of 110–125 g/l and reticulocyte count within normal limits with darbepoetin 25–50 μg weekly. Table 1 shows the results of anti-erythropoietin antibody titers assayed by three different laboratories. RIP (radio-immunoprecipitation assay) and BIACORE assays have a lower limit of sensitivity at 10–100 ng/ml and detect antibodies capable of binding erythropoietin. The less sensitive neutralization assays (bioassays)

Table 1. Anti-epoetin antibody assays performed by three different laboratories in case 2

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Johnson and Johnson</th>
<th>Amgen</th>
<th>Casadevall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen</td>
<td>epo alfa</td>
<td>epo alfa</td>
<td>epo alfa</td>
</tr>
<tr>
<td>Test</td>
<td>RIP</td>
<td>Neutralization</td>
<td>BIAcore</td>
</tr>
<tr>
<td>14.03.2001</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>28.09.2001</td>
<td>Positive</td>
<td>Positive</td>
<td>980</td>
</tr>
<tr>
<td>04.12.2001</td>
<td>720</td>
<td>Positive</td>
<td>810</td>
</tr>
<tr>
<td>23.11.2001</td>
<td>170</td>
<td>Negative</td>
<td>190</td>
</tr>
<tr>
<td>29.03.2002</td>
<td>980</td>
<td>Negative</td>
<td>100</td>
</tr>
<tr>
<td>02.04.2002</td>
<td>Positive</td>
<td>Negative</td>
<td>80</td>
</tr>
<tr>
<td>27.06.2002</td>
<td>100</td>
<td>Negative</td>
<td>110</td>
</tr>
<tr>
<td>26.09.2002</td>
<td>80</td>
<td>Negative</td>
<td>100</td>
</tr>
<tr>
<td>27.11.2002</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>26.03.2003</td>
<td>80</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

In neutralization assays, positive indicates complete inhibition by antibodies of proliferation of cultured erythroid progenitor cells. Detection limit for Amgen BIAcore is 80 U for epo alfa, 100 U for darbepo alfa. For the Casadevall method see Casadevall et al. [4,9] and for BIACORE see Mason et al. [11].
reveal biologically relevant epoetin antibodies, which totally inhibit the proliferation of cultured erythroid progenitor cells [4,9]. Positive indicates complete inhibition, with no colonies formed, and negative indicates no inhibition of proliferation, i.e. lack of neutralizing antibodies in the sample. Amgen BIACORE [11] assay utilizes a biosensor surface into which epoetin alfa or darbepoetin beta may be immobilized. Introduction of serum containing antibodies results in binding of antigen and antibody, which causes an increase in the refractive index (responsive unit) on the surface of the chip. Antibody levels in Table 1 are given as response units, which correspond to certain antibody concentrations in the standard curve drawn. The BIACORE system enables the identification of true cross-reactive antibodies, as pure epoetin alfa and darbepoetin alfa can be tested separately for binding to antibodies in serum samples.

Discussion

These two type 2 diabetic dialysis patients are hitherto the only affirmed cases of epoetin-associated PRCA in Finland. They manifested a full-blown PRCA with frequent red blood cell transfusion requirement after 12 and 8 months of subcutaneous administration of an rHuEPO preparation (epoetin alfa; Eprex®, Johnson and Johnson) but responded favourably to another preparation during (case 1) or after (case 2) immunosuppression with cyclosporin A. In the first case, the other epoetin preparation was administered subcutaneously (epoetin beta; NeoRecormon®, Roche), in the second case, intravenously (darbepoetin alfa; Aranesp®, Amgen). The response has lasted at the moment for 25 and 18 months, respectively.

In our first case, the other epoetin preparation was administered simultaneously with cyclosporin A and started immediately after discontinuation of epoetin alfa. The response in erythropoiesis in this case appeared gradually while it was immediate in our second patient, who received cyclosporin and the other epoetin preparation in succession, darbepoetin starting 8 months after discontinuation of epoetin alfa. Antitythropoietin antibody testing was not performed in the acute phase of PRCA in case 1. The test for neutralizing antibodies was rather inconclusive at the time when the bone marrow had recovered.

In our second case, no antitythropoietin antibodies (immunoreactive nor neutralizing) were detected after 4 months of treatment with epoetin alfa, a few weeks before loss of efficacy. However, both immunoreactive and neutralizing antibodies were detected 2 months after diagnosing PRCA in bone marrow aspiration. Neutralizing antibodies against epoetin alfa turned low or negative 8 months after discontinuation of epoetin alfa and a 4-month treatment course with cyclosporin A. At this time, there were still low levels of immunoreactive antibodies, but they did not increase during the next 12 months despite continuing darbepoetin treatment with no concomitant immunosuppression.

The antibodies were found to neutralize darbepoetin in November 2001, 5 months before starting Aranesp, indicating cross-reactivity. The disappearance of these antibodies earlier than epoetin antibodies may be due to the lower sensitivity of the detection method. Precise erythropoietin antibody testing in clinical practice is hampered by the numerous assay methods (immunological and bioassay) with different sensitivities, as well as the uncertain clinical relevance of the antibodies measured. A standardized, rapid, and reproducible assay to accurately measure anti-erythropoietin antibodies is needed.

The optimal management of a patient with epoetin-induced PRCA is not known [1,2,9,10]. Stopping epoetin treatment is strongly recommended. Some patients have recovered spontaneously with time or after renal transplantation. Results with immunosuppressive regimens without transplantation are inconclusive. Some patients have remained transfusion dependent. At the time these cases were encountered, we were not aware of the recommendation of not to rechallenge the patients with another epoetin preparation [10]. Our concern regarding the transfusion dependency of the patients led to the rechallenge. A priori, our aim with antibody testing was to establish the clinical diagnosis, not to compare different methods or laboratories or to elucidate the mechanisms of PRCA. The results in Table 1 are rather consistent, the differences probably being due to different sensitivity and characteristics of the methods, which have not been extensively reported.

We do not believe that any epoetin preparation has a hyposensitizing or another beneficial effect on the basic mechanisms behind PRCA associated with antitythropoietin antibodies. In our cases PRCA has not relapsed despite treatment with another epoetin preparation. The antigenicity of the other preparations seems to have been low in these sensitized patients.

In conclusion, after recovering from epoetin-induced PRCA, the end-stage renal disease patient may still be anaemic and require erythropoietin or blood transfusions. Another epoetin preparation was efficient in these two cases. We cannot draw any conclusions about the possible role of immunosuppression with cyclosporin A in facilitating the favourable response. Nor can we exclude that re-exposure to epoetin alfa would have been well tolerated under immunosuppression. More evidence is obviously needed. Thus, epoetin should be discontinued in case of treatment-associated PRCA. If, after disappearance of neutralizing antibodies, a rechallenge with another epoetin preparation is performed, the patient should be carefully monitored for reappearance of the antibodies.

Acknowledgements. We are indebted to professor Nicole Casadevall, Service d’Hématologie Biologique, Hôtel-Dieu, Paris, France, as well as to Amgen and to Johnson and Johnson and their Finnish representatives, for organizing and performing the antitythropoietin antibody tests.

Conflict of interest statement. None declared.
References


Received for publication: 7.4.03
Accepted in revised form: 4.12.03