Erythropoiesis-stimulating agents and antibody-mediated pure red-cell aplasia: where are we now and where do we go from here?

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Introduction

Erythropoietin molecules produced by means of gene technology (erythropoiesis-stimulating agents, ESA) have been the agents of choice to correct the anaemia of chronic kidney disease (CKD) since the first of these drugs was licensed in the late 1980s. The side effects seen in the early days may have been due to a too-rapid rise in haemoglobin concentration, along with possible direct effects on non-haematopoietic tissues, including the vascular endothelium. Recently, antibody-mediated pure red-cell aplasia (PRCA) associated with the administration of ESAs has been identified as a serious problem. The number of cases reported has risen from four in the period from 1988, when human recombinant erythropoietin was first introduced to the market, through 1997, to over 100 cases in the last 3 years.

To date, cases of PRCA have been predominantly, though not exclusively, associated with a single brand of ESA, Eprex®. Currently, PRCA is a rare but serious complication and thorough investigation and recommendations on how to deal with the problem are needed.

PRCA

PRCA is a severe, non-regenerative form of anaemia, with selective erythroid aplasia of the bone marrow.
Affected patients have a normochromic, normocytic anaemia, severely reduced reticulocyte count, normal white cell and platelet counts and virtually no erythroid precursors in bone-marrow aspirates. In adults, PRCA is usually an autoimmune disorder in which immunoglobulin (Ig) G antibodies or cytotoxic T-lymphocytes are directed against erythroid progenitors or precursors [1,2]. PRCA usually arises spontaneously or in association with thymoma, lymphoid proliferation or an immune-related disorder, such as lupus erythematosus or rheumatoid arthritis [1,2]. It can occasionally occur secondary to certain drugs or to viral infections, such as B19 parvovirus or hepatitis B virus. In rare cases, PRCA caused by antibodies against endogenous erythropoietin has been reported in patients who have never received ESAs [3].

**PRCA and erythropoietic proteins**

ESA-induced PRCA is caused by the development of neutralizing anti-erythropoietin antibodies in patients undergoing therapy with an ESA. These antibodies cross-react with the patient’s endogenous erythropoietin and lead to an anaemia that is more severe than before the onset of erythropoietic therapy.

As with all therapeutic proteins, the potential for immunogenicity with ESAs was recognized and tested for during development. There was little evidence of any immunogenic effect in clinical trials over the first decade of clinical use. In fact, there were only four reports of antibody-mediated PRCA after use of ESAs in CKD patients from their initial introduction in 1988 to 1997. These reported cases occurred between 1992 and 1997 [4–7].

In 2002, Casadevall et al. [1,8] reported a sudden rise in the incidence of antibody-mediated PRCA after ESA use between 1998 and 2001. Initially, 13 cases [11 epoetin alfa (Eprex®), one epoetin beta and one epoetin alfa and beta] were discussed, followed by 21 cases (the extra eight cases were all treated with Eprex®). All patients had received the drug subcutaneously (s.c.). Severe anaemia resistant to recombinant human erythropoietin (rHuEpo) developed after 3–67 months (median: 7 months) of treatment. Erythropoietic therapy was withdrawn in all patients once the presence of antibodies was confirmed.

In six of the 13 patients, erythropoiesis recovered within 2 years of the diagnosis of PRCA, after receiving immunosuppressive therapy or a renal allograft with concomitant immunosuppression. At the time of publication, no red-cell transfusions had been required for between 9 and 15 months. Immunosuppressive therapy consisted of corticosteroids alone (three patients), corticosteroids with cyclosporin (one patient), cyclophosphamide and corticosteroids (one patient), immune globulin, plasmapheresis and corticosteroids (one patient) and immune globulin and corticosteroids followed by kidney transplantation (one patient).

Three of the seven patients who did not regain any erythropoietic function (one on immune globulin, one on corticosteroids alone and one not receiving immunosuppressant therapy) were still transfusion-dependent >2 years after diagnosis of PRCA. The remaining four patients (two on corticosteroids alone, one on corticosteroids and immune globulin and one receiving no immunosuppressant therapy) were still receiving transfusions as of September 2001, but the authors considered that the follow-up periods of 7–16 months from diagnosis were too short to draw conclusions on clinical outcome.

Antibody titre slowly decreased in all patients after rHuEpo was stopped. Immunosuppressants appeared to hasten the decline in antibody levels and may have allowed erythropoiesis to recover to a level maintained prior to treatment with the ESA [8].

The antibodies induced by the recombinant protein cross-react with both endogenous erythropoietin and other forms of the recombinant protein [2,8–10]. Antibodies identified in patients who developed PRCA after receiving epoetin alfa recognized only conformational epitopes. However, antibodies identified in the single patient described by Casadevall et al. [8], who developed PRCA after receiving exclusively epoetin beta, bound to both conformational and linear epitopes of erythropoietin. Investigation of antibodies from later cases of PRCA following treatment with epoetin alfa suggests that the antibodies are IgG in origin, subclass G1 or G4 [http://www.renux.dmed.ed.ac.uk/EdREN/epo].

**Current situation**

The US Food and Drug Administration received reports of 82 cases of PRCA between July 1997 and December 2001 [11]. Most of the patients (78) were on Eprex®, four were on Epogen® and none was on Procrit®. The high number of cases with Eprex® does not merely reflect increased distribution of this product. From 1997 to 2001, distribution of Eprex® syringes and vials rose from 16.8 million to 30.9 million, while combined distribution of Epogen® and Procrit® syringes and vials rose from 23.1 million to 35.1 million [11]. The median age of affected patients in this period was 61 years for women and 66 years for men. The average length of epoetin treatment before a diagnosis of PRCA was made was 7 months (range: 1 month–5 years).

The incidence and distribution of cases of antibody-mediated PRCA, as reported by manufacturers of erythropoietic proteins, are given in Table 1.

**Possible mechanism of immunogenicity**

Endogenous erythropoietin is extensively glycosylated and this glycosylation is essential for its biological activity in vivo. rHuEpo, manufactured in Chinese
hamster ovary cells, is a 30 400 Da, 165-amino-acid glycosylated protein hormone. The molecule is 60% amino acid and 40% carbohydrate by mass. The carbohydrate portion of the molecule is found on one O-linked and three N-linked oligosaccharide chains. These chains are typically terminated with the negatively charged sugar molecule, sialic acid. Epoetin alfa has more sialic acid residues than epoetin beta [8,12]. The recently licensed ESA darbepoetin alfa differs from rHuEpo in that it contains five N-linked oligosaccharide chains and has a molecular weight of 37 100 Da with a carbohydrate composition of ~51%. These extra chains are accommodated by amino-acid substitutions at five positions along the 165-amino-acid backbone [13]. The antibodies investigated by Casadevall et al. [3] were found to be directed against the protein itself rather than the carbohydrate moiety. This was demonstrated when removal of sugar by enzymatic digestion was seen to have no effect on the affinity of the antibody for erythropoietic protein. This argues against differences in glycosylation as the cause of immunogenicity [9].

Host cell contaminants and protein modification have been implicated in the immunogenicity of biopharmaceuticals [14]. All formulations of epoetin alfa, beta and darbepoetin alfa have been consistently manufactured in the same host cells, Chinese hamster ovary cells, though not necessarily in cells derived from the same cell line or from the same cell bank. So far, there is no evidence of protein modification.

Factors other than the degree of divergence between the endogenous and recombinant molecule can influence the immunogenicity of a therapeutic protein [14]. Processes and formulations that allow protein oxidation or aggregation, such as freeze drying, can enhance immunogenicity [14,15]. Johnson & Johnson [http://www.jnj.com/news/jnj_news/1021024_095632.htm] has come to an interim conclusion that removal of human serum albumin from the formulation of Eprex® in 1998 and increased use of s.c. administration (particularly self-administration, with its product handling and storage problems) appear to play a key role in PRCA associated with the use of the Eprex® brand. Replacement of human serum albumin with polysorbate 80 (0.03% concentration) and glycine as stabilizers in the Eprex® formulation may be relevant. Epoetin beta (Neorecormon®) has used polysorbate 20 as a stabilizer since its registration. Darbepoetin alfa (Aranesp®) also uses polysorbate 80 as a stabilizer (at a lower concentration of 0.005%), but without the occurrence of likely cases of PRCA. This could be because of the apparently complex relationship between excipient protein concentration and aggregation [16]. The use of silicon oil as a lubricant in pre-filled syringes, introduced in 1994 has also been considered as a potential cause of increased immunogenicity. More recently, investigations focus on organic compounds leached by the detergent polysorbate 80 from the rubber plunger in Eprex® pre-filled syringes (B. Sharama, personal communication, Johnson & Johnson). The company reports that it has already replaced the rubber plungers with teflon-coated plungers.

Studies of other therapeutic proteins, including interferon, have shown that the likelihood of an immune response is increased when the protein is given s.c. vs intravenously (i.v.) [14,17–19]. For Eprex®, data reported through May 2002, according to the information provided by Johnson & Johnson, indicate that 94.2% of suspected ESA therapy-related PRCA cases with a known route of administration were associated with s.c. administration. Where route of administration data are available, the estimated incidences of PRCA are 0.67 per 100 000 patient-years for i.v. and 20.66 per 100 000 patient-years for s.c. administration [http://www.ukmicentral.nhs.uk/therapeu/drg_inf/drg23-05.pdf].

<table>
<thead>
<tr>
<th>Company</th>
<th>Number of cases</th>
<th>Route</th>
<th>Patient experience years with product (highest possible incidence per 10 000 patient-years)</th>
<th>Number of cases per country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho Biotech</td>
<td>106 (± 67 under investigation)</td>
<td>Solo use: 15</td>
<td>s.c.</td>
<td>1.7 million (1.11)</td>
</tr>
<tr>
<td>Eprex®</td>
<td></td>
<td>Not sole use: 3</td>
<td>s.c.</td>
<td>~650 000 (0.12)</td>
</tr>
<tr>
<td>Roche</td>
<td>5</td>
<td>i.v.: 2</td>
<td>Both: 1</td>
<td>2.4 million (0.02)</td>
</tr>
<tr>
<td>Neorecormon®</td>
<td></td>
<td>s.c.: 1</td>
<td>Both: 2</td>
<td>56 000 (0.5)</td>
</tr>
<tr>
<td>Amgen</td>
<td>4</td>
<td>s.c.: 0</td>
<td>i.v.: 2</td>
<td>0.12)</td>
</tr>
<tr>
<td>Epogen®</td>
<td></td>
<td>Both:</td>
<td>3</td>
<td>0.12)</td>
</tr>
<tr>
<td>Amgen</td>
<td>0</td>
<td>s.c.:</td>
<td>Both:</td>
<td>0.12)</td>
</tr>
<tr>
<td>Aranesp®</td>
<td></td>
<td>3</td>
<td>Both:</td>
<td>0.12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(unlikely to be associated)</td>
<td></td>
<td>Netherlands: 1</td>
</tr>
</tbody>
</table>

The information is taken directly from letters sent by the companies concerned in response to a request by the President of the ERA–EDTA. The wording, number of cases, routes of administration and distribution information are as provided by the companies.
Testing for antibody-mediated PRCA

Antibody testing is the key to determining whether PRCA is caused by ESA therapy [2]. To date, there is no standardized routine method of testing for antibodies to ESAs. Available assays are either binding assays or bioassays. Bioassays are the only assays that can determine the neutralizing capacity of antibodies. The binding assays in use include radioimmuno-precipitation (RIP), used by Casadevall et al. [8], and enzyme-linked immunosorbent assays (ELISAs). Although direct comparisons of assay methodology have not been published, RIP appears to be more reliable, as ELISAs can have both lower sensitivity and lower specificity. Although Amgen, Ortho Biotech and Roche have all offered to test serum of patients in whom PRCA is suspected, testing by independent laboratories is preferable. Screening tests for anti-erythropoietin antibodies are only recommended in the context of controlled scientific investigations. In routine clinical practice there is no need to test patients with resistance to ESAs, but who exhibit normal bone marrow and have no evidence of PRCA. Figure 1 shows a suggested clinical algorithm for deciding when to test for antibodies associated with ESA therapy.

Actions so far

The labelling of Eprex® has been changed in the European Union to state that in patients with CKD, the product must be administered by the i.v. route only. This appears to be a sensible move as, according to current information, the risk of PRCA with Eprex® s.c. is 2.00 per 10 000 patients compared with 0.06 per 10 000 patients when the drug is given i.v. [http://www.jnj.com/news/jnj_news/1021024_095632.htm]. The labelling of the other erythropoietic proteins has not changed, as current evidence indicates that the risk of PRCA with these products is much lower, by either route, than Eprex®. This does not exclude the possibility, however, that an increase in the incidence of PRCA in response to other s.c. administered brands may be observed in the future. A close follow-up is mandatory. It should also be noted that all protein-based therapeutics are generally sensitive to environmental changes and should be stored and handled according to the product labelling.

In May 2000, Ortho Biotech issued a recommendation for handling Eprex® in hospitals and satellite/outpatient centres, reinforcing the optimum handling and storage conditions so that the stability of the drug is maintained. It will be important to record future cases of PRCA in a way that allows for analysis of whether better handling and storage of the drug correlate with a reduced incidence of PRCA.

Regulatory authorities in the European Union and Canada have published safety notices based on the information from Johnson & Johnson. Most advise that Eprex® should not be given s.c. in patients with CKD and attention should be paid to storing the product at the recommended temperature of 2–8°C. In addition, the possible risks of giving ESAs should be explained to patients and any adverse events reported. They emphasize that ESA therapy must be stopped in patients developing PRCA and patients should not be switched to another brand of ESA. The Canadian health authorities advise that Eprex® should be given i.v. where feasible, but if this is not feasible, any

Fig. 1. Clinical algorithm for investigating suspected cases of PRCA. Adapted from Macdougall [20] with permission.
benefits of administration should be weighed against risk before giving the product s.c.

The Canadian Society of Nephrology has decided not to advise against using Eprex® s.c., because it considers that substances are either immunogenic or not. They argue that the s.c. route may enhance immunogenicity but not confer it. A small number of cases associated with the i.v. route have been reported and the possibility of more in the future cannot be ruled out. In addition, the s.c. route was used for the administration of Eprex® in the period before 1998 when there were few reported cases of ESA-associated PRCA and other ESAs have also been given s.c. for a long time with few related cases of PRCA.

The Canadian Society of Nephrology does, however, recommend that physicians consider a change from pre-filled syringes of Eprex® to multidose vials or a different brand of ESAs.

The legal implications of not following the recommendations of the national regulatory authorities need careful consideration, especially where alternative formulations without regulatory restriction are available.

To track new cases of PRCA, the ERA–EDTA is organizing an independent PRCA register, possibly using the facilities of the ERA–EDTA Registry in Amsterdam. National registries of confirmed and suspected cases for investigation are also being set up.

Recommendations

Antibody-mediated PRCA is a serious, but fortunately rare, adverse event related to ESA therapy. The subject is still under intense investigation by the regulatory authorities, the manufacturers of ESAs and independent scientists of the nephrology community. More definitive results from these sources are needed before we can make specific, evidence-based treatment recommendations.

Notification of PRCA cases comes from spontaneous reports of adverse events and ad hoc post-marketing surveillance. A more organized and structured system of reporting through the proposed ERA–EDTA and national registries should contribute valuable data. As part of the registry and notification process, strict criteria for case definition and standardized assay procedures need to be specified and implemented.

We believe that the number of reported cases of PRCA in the current situation does not justify withholding treatment with ESAs in patients with CKD. Actions nephrologists can take to limit the effect of this new complication and help to understand causes and risk factors include:

(i) Following the recommendations of the regulatory authorities and reporting suspected cases.
(ii) Being aware of the problem and understanding the signs and symptoms that indicate antibody-mediated PRCA—reticulocyte count is a sensitive and early marker of PRCA.
(iii) Treating any patient who does not respond as expected to ESAs as a possible case of PRCA, after evaluating other, more common causes of unresponsiveness, such as iron deficiency, blood loss, infection and inflammation and following the algorithm for investigating suspected cases of PRCA (Figure 1).
(iv) Being aware that the antibodies are neutralizing and will cross-react with all currently available ESAs and endogenous erythropoietin: all erythropoietic therapy must be stopped immediately for patients where immunogenicity is suspected [10].
(v) Considering obtaining patients’ permission for a blood sample to be taken and stored before switching brands or route of administration. This sample may be used as a baseline sample in case PRCA should develop.

Whatever the outcome of any new, intensive research, antibody-mediated PRCA will probably continue to have an impact on the use of ESAs in clinical practice, not least from the standpoint of increased costs related to switching Eprex® to the i.v. route in line with the regulatory authorities’ new regulations. Finally, it is important to note that as patents on currently licensed ESAs expire, safety implications of generic compounds may also become a major issue.

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Conflict of interest statement. The authors declare that all of them are members of Advisory Boards and/or speakers for almost all the three companies involved in the field of erythropoietic agents.

References

Which dialyzer membrane to choose?

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Keywords: bioincompatible; cellulose; dialyzer; high-flux; low-flux; synthetic

Introduction

Exchanges through dialyzer membranes aim: (i) at the removal of uraemic solutes that are retained because of renal failure (e.g. urea) and (ii) at the restoration of depleted compounds (e.g. bicarbonate).

The originally used cellulosic membranes were derived from cotton and therefore named ‘natural’. They activated complement and leukocytes, inducing an inflammatory reaction as one of the indices of ‘bioincompatibility’ [1]. Later on, chemically developed ‘synthetic’ polymers appeared to mitigate this activation [2]. Furthermore, masking hydroxyl groups, which are responsible for the complement activation with cellulosic membranes, also resulted in more biocompatibility [3]. Therefore, cuprophan and its analogues were called ‘unmodified cellulosic’ vs the more biocompatible, later developed ‘modified/regenerated cellulosic’ membranes.

Many synthetic membranes have large pore sizes allowing higher rates of water flux and permitting a higher ultrafiltration capacity as well as a better removal of high molecular weight ‘uraemic solutes’ than membranes with smaller pore size. Therefore, although a high ultrafiltration rate and the capacity to remove large molecules do not strictly run in parallel, large pore membranes are mostly referred to as ‘high-flux’, in contrast to ‘low-flux’ membranes with smaller pores. Five general types of membranes are available at present (Table 1).

In this review, the most relevant membrane characteristics allowing a rational choice for treatment are discussed. More extensive relevant data can be found in the European Best Practice Guidelines for haemodialysis, part I [4,5].

Relevant membrane characteristics

Biocompatibility towards leukocytes and the complement system

Biocompatibility describes materials, which cause only minor biochemical and biological effects. In this review,