Antibody-mediated pure red cell aplasia (PRCA): epidemiology, immunogenicity and risks

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Abstract

Although epoetin-induced antibody (Ab)-mediated pure red cell aplasia (PRCA) was very rare prior to 1998, a large increase in the number of global cases was observed from 1999 to 2002 in patients treated with erythropoiesis-stimulating agents (ESAs) for the anaemia associated with chronic kidney disease (CKD). The number of global cases of this immunological form of PRCA has declined precipitously since 2003 following increased awareness of this disorder and changes in the handling and administration of the formulation of epoetin-α (EPO) that was associated with the majority of cases. Current recommendations state that patients should stop treatment immediately following a diagnosis of Ab-mediated PRCA and not resume treatment with the same or another ESA. The feasibility of re-treatment following remission from PRCA or during ongoing immunosuppressive therapy is currently under investigation. The immunological mechanism for developing Ab-mediated PRCA is unknown, but a variety of factors may increase the immunogenicity of epoetin or other ESAs. Product-related factors that have the potential to impact on immunogenicity include sequence variations in proteins, the degree and nature of protein glycosylation, the manufacturing process, handling and storage, and components and properties of the product formulation. Patient-related factors associated with developing Ab-mediated PRCA include skin reactions, immune status and treatment history. Increased awareness among physicians of the factors contributing to the development of PRCA and its distinguishing clinical features has coincided with studies aimed at identifying effective therapies for this disorder. A number of immunosuppressive therapies have been shown recently to reconstitute effectively the erythropoietic response in patients with PRCA. However, therapeutic approaches for this serious immunological reaction to recombinant ESAs remain investigative.

Keywords: epidemiology; erythropoietin; pure red cell aplasia

Introduction

Pure red cell aplasia (PRCA) is a relatively rare haematological disorder that leads to a progressive, severe anaemia. This disorder has been historically associated with a congenital disorder (Diamond-Blackfan syndrome) [1] or as an acquired disorder such as in relation to a thymoma, systemic lupus erythematosus or a viral infection [2]. An immunological form of PRCA, antibody (Ab)-mediated PRCA, has been recognized in patients receiving recombinant erythropoiesis-stimulating agents (ESAs) including human erythropoietin (epoetin, EPO) as an alternative treatment to transfusions in CKD [3,4]. This type of PRCA is caused by the development of Abs that neutralize all exogenous epoetin preparations and cross-react with endogenous EPO, resulting in EPO-resistant anaemia and transfusion dependence. These Abs are characteristically produced in patients within an average treatment period of 9 months [5]. Of interest, Ab-mediated PRCA has not been documented in cancer patients receiving ESAs for chemotherapy-related anaemia.

Because of its immunological origin, Ab-mediated PRCA requires some form of immunosuppressive therapy to suppress Ab production and promote restoration of normal erythropoietic activity. Two distinguishing features of Ab-mediated PRCA are the associated decline in blood haemoglobin (Hb) level of ~40 g/l per month, and a decrease in the number of circulating reticulocytes to <10 000/μl of blood [6]. The immunological mechanisms underlying the development of Ab-mediated PRCA in patients with CKD...
remain to be determined, and therapies for treating this disorder are being investigated.

Here we review the epidemiology of Ab-mediated PRCA including the temporal increase and decrease in the number of global cases since 1988, the different immunogenic characteristics of currently available ESAs, and the patient- and product-related risk factors for developing PRCA.

Epidemiology

The emergence of Ab-mediated PRCA was unexpected. Initially described in only a few isolated case studies published prior to 1998, there was a notable increase in the number of reported cases of this condition between 1999 and the end of 2002. This increase in case numbers was followed by a dramatic decrease in reported cases during 2003 and 2004 (Figure 1). Although the immunological mechanisms for Ab-mediated PRCA remain unclear, various clues have emerged regarding its aetiology. First, the majority of reported cases of this type of PRCA are associated with the use of a single preparation of epoetin-α (Eprex®, Erypo®, Ortho Biotech, LLC, Manati, Puerto Rico) [7] distributed outside the USA, particularly in Europe [5, 8, 9]. This product was reformulated in 1998 in response to European regulatory guidance on the use of plasma-derived products for formulation of medicinal products [10, 11]. This manufacturing change removed human serum albumin (HSA) from the Eprex® formulation, and replaced it with Tween-80 (polysorbate 80), an amphipathic surfactant used commonly as a stabilizer in pharmaceutical products. Secondly, all patients with PRCA associated with the use of Eprex® had received subcutaneous (s.c.) administration of this product that was packaged in single-use plastic syringes [6]. Thirdly, all patients that developed Ab-mediated PRCA following treatment with multiple ESAs, including another epoetin-α (Epogen®, Procrit®, Amgen Inc., Thousand Oaks, CA/Johnson & Johnson, New Brunswick, NJ) [12] or epoetin-β (NeoRecormon®, Roche Pharmaceuticals, Mannheim, Germany) [13], were exposed to Eprex® at some point during the course of their treatment.

Prevalence and regional distribution of cases

Since 1988, there have been 206 confirmed cases of antibody-mediated PRCA worldwide. Figure 1 illustrates the global case numbers of Ab-mediated PRCA following exposure to Eprex® alone or in combination with another ESA prior to 1998, until August 31, 2004, as reported by Johnson & Johnson in October 2004 [9]. These data clearly show that the majority of cases have occurred since 1999, with the largest number of reported cases occurring in 2002. From 1988 until August 31, 2004, 183 cases of Ab-mediated PRCA were reported in patients with CKD receiving Eprex® alone and 23 cases with the use of Eprex® plus another ESA [9]. From January to August 2004, there were only three reports of cases of Ab-mediated PRCA with the use of Eprex® alone or with another ESA.

The majority of epoetin-induced cases of PRCA have been reported outside of the USA, with apparent regional differences in incidence among European nations. For example, since 1998, France had 33 reported cases, the UK had 26 cases and Germany had eight cases, whereas Australia had 12 reported cases [6, 14, 15]. A separate report, published in 2003, cited 15 cases of epoetin-induced PRCA in Singapore [16]. Regional variations in incidence may be due to differences in the preferred route of product administration and storage and handling practices [17].

Product-associated incidence of Ab-mediated PRCA

Recent new updates from major drug manufacturers and SwissMedic have reported the product-associated incidence of Ab-mediated PRCA [9, 18, 19]. The exposure-adjusted incidence rates of Ab-mediated PRCA for the different available ESAs during the
peak incidence years of 2001–2003 are shown in Figure 2.

These data clearly show that the majority of PRCA cases were associated with administration of the Eprex® brand of epoetin-α. Recent reviews by Bennett and colleagues, and Cournoyer and colleagues [20,21], list calculated incidences per 100 000 patient-years of experience: (i) 18 cases for Eprex® without HSA (Bennett) vs 26.9 cases (Cournoyer); (ii) six cases for Eprex® with HSA (Bennett) vs 2.2 cases (Cournoyer); (iii) one case for Neorecormon® (Bennett) vs 1.6 cases (Cournoyer); (iv) 0.2 case for Epogen®/Procrit® (Bennett) vs 0.4 cases for Epogen® (Cournoyer) and 0.7 cases for Procrit® (Cournoyer); and (v) no cases for Aranesp® (Cournoyer).

In Europe, where most cases have occurred, there were the aforementioned 183 cases of Ab-mediated PRCA with the s.c. use of epoetin-α (Eprex®) alone and eight cases with the use of epoetin-β (NeoRecormon®) alone. In the USA, six cases have been reported with use of the Epogen® (n = 4)/Procrit® (n = 2) brands of recombinant epoetin-α [18]. The low number of PRCA cases associated with NeoRecormon® and Epogen®/Procrit® may represent a background level of cases in large patient populations. Up to March 31, 2004, there were no reports of Ab-mediated PRCA in patients treated with darbepoetin-α (Aranesp®) alone; six cases have been reported in patients that received darbepoetin-α combined with another ESA [18].

In the last 2 years, there has been a significant worldwide decrease in newly reported cases of Ab-mediated PRCA in patients with CKD receiving ESAs, particularly in countries where labelling recommendations have been followed [17]. Based on the Johnson & Johnson website, the worldwide incidence of PRCA in patients receiving Eprex® has dropped from 4.47 cases per 10 000 patient-years in 2002 to 0.63 cases per 10 000 patient-years in the first 8 months of 2004. This translates into only three cases of Ab-mediated PRCA associated with the use of Eprex® from January to August 2004; two of these cases were in patients treated with epoetin-α only [9].

Formulation and immunogenicity of various erythropoietin products

The introduction of genetic recombinant technology made it possible to produce therapeutic products that were identical or nearly identical to native human proteins [22]. However, the immunogenicity associated with human and non-human sources of protein is an inherent threat to the use of biopharmaceutical agents such as the ESAs. The consequences of Ab formation during treatment with biopharmaceutical agents vary widely: some Abs may be clinically insignificant, whereas others may cross-react with the endogenous protein, as in the case of epoetin, and inhibit or neutralize its activity. A variety of factors influence the immunogenicity of biopharmaceuticals, including the genetic make-up of the patient, sequence variations in proteins, glycosylation, the manufacturing process, handling and storage, and components and properties of the formulation itself [22].

Attention has been focused recently on how one brand of epoetin-α may have become more immunogenic than other contemporary ESAs. Several hypotheses have been put forward to explain this increased immunogenicity, including route of administration, storage and handling issues, the possible deleterious effects of polysorbate 80 on protein stability, and the presence of leachates from the rubber plungers used in pre-filled syringes. For example, the s.c. route of administration is known to elicit a greater number of immunological responses to proteins than the intravenous (i.v.) route [22], and no cases of PRCA have been described following only i.v. administration of Eprex®. In 2002, the regulatory authorities in Europe recommended switching patients from the s.c. route to the i.v. route of administration for Eprex® [10]. Following these recommendations, the total global number of cases of Ab-mediated PRCA has declined sharply [9]. Other hypotheses include altered product stability or biochemical structure caused by the formation of aggregates or micelles, or improper folding of protein in product vials or syringes [4,23]. However, these hypotheses remain unproven.

Fig. 2. Global incidence rate of antibody-mediated PRCA by product, 2001–2003 [9,18,19].
Stabilizers

One of the major differences among the commercially available epoetins is formulation. Proper formulation ensures stability and optimal activity of therapeutic proteins that may be present in low concentrations or susceptible to degradation. Product stability can be increased with the use of certain stabilizers. A common ingredient that is added to many products as a stabilizing agent is HSA, which is considered to be non-immunogenic because of its antigenic similarity to endogenous HSA. The epoetin-α preparations Epogen® and Procrit® contain HSA as a stabilizer [12,24]. However, HSA was removed from the formulation of epoetin-α (Eprex®) in 1998, and replaced with polysorbate 80 and glycine in pre-filled syringes distributed outside the USA [8,9]. The epoetin-β formulation (NeoRecormon®) differs from the epoetin-α formulations in that it contains a combination of stabilizers, including polysorbate 20, glycine, a mixture of five other amino acids, urea and calcium chloride [13,25]. Darbepoetin-α (Aranesp®) is supplied in single- or multi-use vials containing either of two different formulations using phosphate-buffered saline plus 0.05 mg/ml of polysorbate 80 or 2.5 mg/ml of HSA stabilizer [26]. In addition to these factors, various ESAs are available in different preserved forms, which may also affect the stability of products under different or adverse environmental conditions. For example, epoetin-β (NeoRecormon®) was previously supplied as a lyophilized powder that required rehydration immediately prior to administration, while Eprex® and Epogen®/Procrit® are supplied as aqueous solutions. Proper storage during shipping and in formularies is emphasized in the prescribing information of these products.

Leachates

A hypothesis has been investigated recently which postulates that the observed increase in immunogenicity of epoetin-α is due to the formation of leachates from the rubber plungers of pre-filled syringes [23]. Preliminary data suggest that leachates may form in pre-filled syringes of epoetin-α that contain polysorbate 80 stabilizer instead of HSA [23]. These leachates include low molecular weight elastomers, curing agents, antioxidants and plasticizers. The results of additional experiments showed that a combination of epoetin-α, leachates and silicone oil (plunger lubricant) elicited the production of anti-EPO Abs in experimental mice [23]. Further research into this interesting hypothesis is ongoing.

Micelles

It has been postulated that the high concentration of polysorbate 80 contained in the Eprex® brand of epoetin-α can cause the formation of micelles [27]. Epoetin molecules, thought to be integrated into the surface of these micelles, may thus form immunogenic complexes [27,28]. It is possible that micelles are dispersed by i.v. administration but sequestered at the sites of s.c. administration [27]. However, no studies to date have demonstrated the presence of micelles or insoluble aggregates in available ESAs. Therefore, it remains to be determined if micelles or aggregates can contribute to the immunogenicity of ESAs.

Handling and storage issues

The differences in formulation among the commercially available epoetins pose different handling and storage requirements [25]. For example, heat, sunlight and other stressful environmental conditions may cause denaturation of proteins. In turn, partial denaturation of proteins may increase their potential immunogenicity [22]. Recommended storage and handling requirements may present certain limitations for patients, who often self-administer their epoetin. Following the increase in cases of Ab-mediated PRCA, the labelling of Eprex® was changed in the European Union to mandate that this product be administered by the i.v. route only. In May 2000, Ortho Biotech issued a recommendation for hospitals and outpatient departments to emphasize the importance of optimum handling and storage of Eprex® to maintain drug stability [29].

Patient factors associated with Ab-mediated PRCA

Skin reactions

Skin reactions in patients treated with epoetins may be an early indicator of an increased risk for Ab-mediated PRCA, although such reactions have not been observed in the majority of cases. Weber and colleagues reported a case history of a patient with anti-EPO antibodies, in whom i.v. injection of different epoetins evoked skin reactions at the sites of previous s.c. injections, indicating the local persistence of sensitized cells [30]. A second patient that developed Ab-mediated PRCA following s.c. administration of epoetin-α was reported in 2003 by van Paassen and colleagues [31]. These reports provided evidence that, in some cases, cutaneous reactions at injection sites may reflect immune sensitization to the treatment protein, which can precede the onset of PRCA development by several weeks. Moreover, continuation of epoetin therapy in patients with anti-EPO Abs carries the risk of anaphylactoid reactions similar to those reported in the Weber and van Paassen studies.

Immune status

It has been observed that most patients who developed Ab-mediated PRCA did not have a history of
autoimmune disease or of drug-induced immune reaction [4]. Nevertheless, aberrant immune reactivity to recombinant proteins such as EPO may occur in a small percentage of treated patients. The potential immunogenicity of recombinant ESAs appears to be associated more with the product formulation and route of administration than with a patient’s underlying immune status.

As part of an effort to determine the role of pre-existing Abs in the development of PRCA, the relative concentrations of anti-EPO Abs (baseline and post-treatment) were measured in 1501 patients enrolled in darbepoetin-α clinical trials using a validated BIAcore 3000 immunoassay [32]. This exercise was also done to establish the usefulness of the BIAcore assay as a screening method for accurately measuring anti-EPO Abs. The majority of patients received darbepoetin-α by the s.c. route of administration. In this study, low levels of non-neutralizing baseline Abs that bound to epoetin-α or darbepoetin-α were detected in 59 (4%) patients prior to administration of darbepoetin-α; 21 were EPO-naive and 38 had received prior EPO treatment. However, these baseline Abs were not associated with the development of Ab-mediated PRCA in any patient and did not neutralize the biological activity of EPO. Therefore, the BIAcore assay proved useful as a method to screen patients for anti-EPO Abs but was not considered to be a good surrogate marker for Ab-mediated PRCA. Although it is not clear if patients who are positive for anti-EPO antibodies at baseline ultimately develop PRCA, screening of EPO-resistant patients and careful monitoring of Ab-positive patients may reduce overall case numbers.

Product factors associated with Ab-mediated PRCA

Treatment history

Recent data provided by Johnson & Johnson on Eprex®-related cases of Ab-mediated PRCA where the route of administration is known indicate that ~94% of patients developing this disorder had received epoetin-α by the s.c. route [29]. Other factors in relation to Ab-mediated PRCA include an initial erythropoietic response to treatment (6–18 months) with a stable blood Hb level, followed by a sudden decline in circulating reticulocytes to <10,000/mm³, resistance to the same or increased dosage of epoetin or other ESAs, transfusion dependence and a rapid increase in serum transferrin saturation and ferritin concentration [4,6]. In all confirmed cases, anti-EPO Abs were detected by a sensitive Ab assay, such as the radio-immunoprecipitation (RIP) assay [6,33].

Glycosylation

The degree of biochemical divergence between endogenous proteins and their recombinant versions can potentially influence the immunogenicity of the recombinant molecules [29,34]. Endogenous EPO and recombinant ESAs are extensively glycosylated, which is essential for their biological activity in vivo. Recombinant EPO is composed of the same amino acid sequence as endogenous EPO, but its glycosylation varies [35]. Endogenous EPO bears four glycosylation sites that carry three N-linked and one O-linked oligosaccharide chain [35]. Specifically, epoetin-α, -β and -σ contain the same number of N- and O-glycosylation sites; however, they contain a higher proportion of sialylated, acidic carbohydrate residues than endogenous EPO [36]. Darbepoetin-α contains two additional N-linked carbohydrates, increasing its potential maximum number of sialic acid residues from 14 up to 22 [37]. Of interest, the results of recent in vitro and experimental immunization studies have shown that increased glycosylation of epoetin decreases the potential for Ab production [38], although the carbohydrate moieties are not immunogenic themselves [3].

Achieving beneficial outcomes with Ab-mediated PRCA

A better understanding and description of the characteristic signs and symptoms of Ab-mediated PRCA has raised awareness of this disorder among nephrologists and other physicians. Moreover, recommendations for switching the administration of Eprex® from the s.c. to the i.v. route correlates with a reduced overall incidence of Ab-mediated PRCA [9,10]. This supports the aetiological importance of s.c. administration to the ultimate development of PRCA in affected patients.

Recent studies have shown that immunosuppressive therapy hastened the disappearance of circulating Abs in patients with EPO-induced PRCA, and allowed endogenous erythropoiesis to recover to pre-treatment levels [6,33]. In a retrospective study of 47 patients who developed PRCA during EPO therapy (primarily Eprex), 29 of 37 patients (78%) who received immunosuppressive therapy recovered, whereas none of the nine patients who did not receive immunosuppressive therapy recovered [6]. Red blood cell production recovered only when patients received immunosuppressive therapy. The recognition of Ab-mediated PRCA in patients treated with recombinant epoetins has underscored the need for full clinical documentation and post-marketing surveillance with newer ESAs and biosimilar products, as well as therapeutic recombinant proteins in general [39].

Conclusions

Ab-mediated PRCA exemplifies a serious immunological reaction to a recombinant erythropoietin protein. The recent increase in the number of reported cases of
this disorder in EPO-treated patients with CKD presents a treatment dilemma for physicians and their patients. Risk factors for Ab-mediated PRCA include s.c. administration of certain formulations of epoetin-α, as well as unknown patient factors. Although treatment approaches exist for managing Ab-mediated PRCA, these therapies remain investigational in nature. Careful monitoring for the presence of anti-EPO Abs that coincide with a sudden and rapid decline in blood reticulocyte counts and Hb level should help in identifying those patients at risk for developing PRCA.

Note added in proof: As of March 2005, 2 suspected cases of PRCA in patients treated with darbepoetin-alpha alone are under investigation.

Conflict of interest statement. The author has received honorarium and/or research grants from Amgen, Roche, and Ortho Biotech.

References

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