The (re)challenging question of erythropoiesis-stimulating agents inducing pure red cell aplasia

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Introduction

The introduction of recombinant human erythropoietin (EPO) into routine clinical practice in the late 1980s heralded a new dawn for the treatment of anaemia in chronic kidney disease (CKD). Apart from the financial burden, limiting its use in the developing world, the early side-effect profile was mild. It has been shown that full correction of anaemia, requiring high doses of EPO, does not confer mortality benefit to patients suffering from CKD [1,2], and is potentially detrimental to survival [1] even in haemodialysis patients with cardiac disease; the administration of EPO to raise haematocrit to 42% is not recommended [3].

Pure red cell aplasia (PRCA), secondary to EPO, will form the basis of this review. This idiosyncratic immunological reaction involves the generation of anti-EPO antibodies (Abs) resulting in significant anaemia. The mechanisms governing this immunological response remain only partially understood. Whilst PRCA is infrequent, the disease is severe, and the aggressive treatment requirements present a significant complication to patients and a therapeutic conundrum to the treating clinician.

History of PRCA

PRCA is a rare haematological disorder characterized by an absence of bone-marrow erythroid precursors (erythroblasts) and associated reticulocytopaenia (\(<10 \times 10^9/L\)) [4]. It manifests as significant anaemia, rendering patients transfusion dependant. Patients develop Abs (IgG 1/IgG 4) directed against the protein moiety of the EPO molecule [5]. These Abs neutralize all currently available erythropoiesis-stimulating agents (ESAs) as well as the patient’s endogenous EPO.

Whilst reports of EPO-associated Ab-mediated PRCA were rare prior to 1998, possibly partly explained by underreporting, there was a sharp increase in reported cases occurring during the period 1999–2002 (reviewed) [6]. This sharp increase in reports was mainly limited to the UK, Europe and Canada [7] and was almost exclusively associated with the subcutaneous (s.c.) administration of epoetin alpha (EPREX®/ERYPRO®; Ortho Biotec, Janssen-Cilag, NJ, USA), although all available ESAs are associated with this adverse reaction. The increased occurrence of PRCA with s.c. EPREX facilitated the (temporary) withdrawal of the license for the s.c. administration of EPREX and subsequently a recommendation that ESAs be administered intravenously, if feasible, to these patients.

The most common initial explanation offered for this occurrence centred on the substitution of human serum albumin for Tween-80 as a stabilizer in epoetin alpha preparations and the use of uncoated rubber syringe stoppers in pre-filled syringes during manufacture. Animal [8], chemical [8,9] and epidemiological [10] investigations have been used to support a hypothesis that leachates from the stoppers served as immunological adjuvants promoting these adverse reactions. This argument was strengthened by the decline (>80%) in reported cases of (EPREX-associated) Ab-mediated PRCA observed after alterations to drug preparation (Teflon coating of the rubber stopper) and delivery (intravenous) [7]. Interestingly the animal data did not demonstrate an immunological response when the leachates were given with EPO, but only with the known antigen, ovalbumin. Further published criticisms of the animal data include the criticisms of research methodology and its description, inadequate controls and the method of publication [11].

An alternate explanation offered is that micelle aggregations in the EPREX solutions are responsible for breaking ‘B cell tolerance’ [12,13]. Improvements in the cold chain could potentially explain the decreased incidence
post-2002. Whilst much of this evidence remains speculative, it does offer an alternative explanation for the divergent immunological responses seen. It is feasible that the answer will be multifactorial: handling, cold chain, s.c. administration, change in formulation, all contributing. In conclusion, there remain unanswered questions as to why a (relatively) minor change in formulaic preparation resulted in such a catastrophic scenario, with cases abruptly declining following the cessation of s.c. EPREX administration.

Treatments for PRCA

Cessation of EPO therapy is universally accepted as first line therapy. Immunosuppression, most commonly with cyclosporine, corticosteroids or cyclophosphamide, has been widely used to halt the overactive immune response with reduction in measurable anti-EPO Abs and stabilization of transfusion requirements. In addition, intravenous immunoglobulin, which has been used successfully in idiopathic PRCA, rituximab [14], plasma exchange and immunoadsorption [15] have all been used with varying success.

In a retrospective analysis of 47 patients suffering from PRCA secondary to ESAs (mostly EPREX, all s.c. administered), a clear benefit for the use of immunosuppression was demonstrated. Ten patients receiving no immunosuppression demonstrated no haematological recovery, whilst 78% treated with immunosuppressive therapy recovered function [16]. These results were substantiated by Bennett et al. who provided long-term (mean 9 months) follow-up from 170 patients suffering from EPO-induced PRCA. One hundred sixty-nine patients had received s.c. epoetin alpha. In total, over one-third of patients recovered haematological function (37%), with significantly higher rates of recovery in those patients receiving one or more immunosuppressive agents (57%), calcineurin inhibitors proving more efficacious than immunoglobulin therapy [17]. Transplantation has been successfully performed on patients suffering from PRCA [18], including those resistant to immunosuppressive therapy, and up to 95% have been reported to recover haematological function after successful transplantation [17].

Significant concerns existed regarding re-challenging patients diagnosed with Ab-induced PRCA with EPO therapy, even after a period of immunosuppression. However, the continuous demands of red cell transfusions for these patients, combined with the obvious difficulties in safe cross-match meant that, in selected cases, risk taking became necessary. The first case of successful re-challenge was reported in 2004 (Finland) [19] followed shortly by a further report from the UK [20]. In this issue of *Nephrology Dialysis and Transplantation*, the long-term success of this treatment regime is described [21]. Clearly for this patient the re-introduction of EPO was successful therapy raising the haemoglobin level whilst eliminating the need for further blood transfusions. The long duration of treatment (4 years) with adjunctive immunosuppression demonstrated that re-introduction offers a potentially safe effective long-term treatment option.

To date there is only one report of relapse of PRCA after re-challenge in the literature, and interestingly in this patient immunosuppression had been terminated [22]. In Bennett’s series, 34 patients have been re-challenged with ESAs and 18 (56%) recovered EPO responsiveness, with the highest recovery rates demonstrated in patients whose Abs had resolved (89%) [17]. This encouraging response allowed the authors to challenge the (currently) existing recommendations suggesting that EPO should not be prescribed to patients suffering from EPO-induced PRCA.

Current recommendations for treating patients with a substantive diagnosis of EPO-induced PRCA would include immediate cessation of the particular ESA and initiation of immunosuppression (steroid + cyclosporine/cyclophosphamide). From the current evidence, re-challenge with an alternative ESA can be considered in those patients with good serological response to immunosuppression [23].

Novel erythropoietin-stimulating agents for treating renal anaemia

Whilst epoetin alpha and epoetin beta have been in clinical practice for over 20 years, followed by darbepoetin alpha for nearly 10 years, there is likely to be a sharp increase in both the number and type of ESAs available for patients with CKD shortly, reviewed recently [24,25]. The first biosimilar of epoetin alpha received marketing approval in August 2007 in Europe. Studies of pharmacodynamics and kinetics showed similar profiles to epoetin alpha and epoetin beta. The use of epoetin alpha as a comparator in these studies means that the biosimilar was restricted to intravenous administration. The possible immunogenicity of the biosimilar and the potential development of PRCA has been considered, and a cohort study has been described, although the numbers of patients involved or the proposed length of the study are unknown, and it is likely to be underpowered to determine risk [26].

Of particular interest are the peptide-based ESAs, which are unrelated in sequence to EPO but bind to the EPO receptor. Hematide (Affymax, Palo Alto, CA, USA) has shown promise in early human clinical trials for treating patients with anaemia, secondary to CKD [27]. Peptide ESAs are not protein based which will mean easier manufacture ensuring a cheaper, more stable and less immunogenic product. In an experimental model of PRCA it was found that EPO Abs do not cross-react with Hemitide and similarly Hemitide Abs do not react to EPO. Furthermore, Hemitide corrected the antibody-induced anaemia in these animals [28]. This encouraging result facilitated a (human) clinical trial examining the potential role of Hemitide as rescue therapy in patients with Ab-mediated PRCA. Provisional results from this study are encouraging demonstrating a benefit from Hemitide, with anaemia correction, without the need for immunosuppression [29] (and personal communication). This may be an attractive management strategy, particularly
in those patients who may not tolerate long-term immunosuppression.

**Conclusion**

EPO therapy has revolutionized the treatment of anaemia associated with CKD. The immunological basis of EPO-induced PRCA has presented an unsolved puzzle to nephrologists. Treatment protocols for patients suffering PRCA are now well accepted and include immunosuppression, with resolution of the anti-EPO Abs and subsequent re-challenging with EPO. Novel ESAs that do not cross-react with EPO Abs offer a potential rescue therapy.

**Conflict of interest statement.** None declared.

(See related article by N. Campbell et al. Update on reintroduction of epoetin in a patient with pure red cell aplasia. *Nephrol Dial Transplant* 2008; 23: 3365.)

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