Epoetin-associated pure red cell aplasia in patients with chronic kidney disease: solving the mystery

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Abstract
A substantial increase in the incidence of pure red cell aplasia (PRCA) associated with recombinant human erythropoietin (epoetin) treatment occurred in 1998. The upsurge of antibody-mediated PRCA was almost exclusively associated with chronic kidney disease patients who received subcutaneous epoetin therapy and the formulation of epoetin-α distributed outside the USA (EPREX®/ERYPO®). A systematic programme of technical, immunological and epidemiological investigations was initiated to identify the possible causes. The potential causes were evaluated on the basis of the following criteria: temporal correlation with the increase in incidence of PRCA, significant difference between EPREX®/ERYPO® and other epoetin products, sufficient concentration in the product to elicit a weak immune response, evidence of immunogenic activity in animals supportive, and consistent with available clinical data. Organic compounds that were leached from rubber stoppers through the action of polysorbate 80 were detected in pre-filled syringes with uncoated rubber stoppers containing polysorbate 80-formulated EPREX®/ERYPO® (introduced outside the USA in 1998). The leachates were not present when the stoppers were coated, in the product formulated with human serum albumin or in other epoetin products. The adjuvant activity of the leachates was demonstrated in mice. The incidence of PRCA was significantly higher in patients exposed to the polysorbate 80 formulation of epoetin-α delivered from pre-filled syringes with uncoated rubber stoppers, which were recalled in 2003, than in patients exposed to the same formulation from syringes with coated rubber stoppers. In conclusion, these data strongly suggest that leachates were the critical contributory factor in the increased incidence of antibody-mediated PRCA attributed to EPREX®/ERYPO®.

Keywords: chronic kidney disease; epoetin; erythropoietin; leachates; polysorbate 80; pure red cell aplasia

Introduction
Over the decade following the introduction of epoetin in 1989, many hundreds of thousands of anaemic patients have received the drug, increasing their haemoglobin levels safely and, generally, with few side effects. There were, however, sporadic reports of patients with chronic kidney disease (CKD) developing epoetin-associated pure red cell aplasia (PRCA) [1–3]. PRCA is a rare disorder that manifests as a severe, isolated anaemia of sudden onset, characterized by an almost complete absence of red cell precursors in the bone marrow and a reticulocyte count <10×10⁹/l [4,5]. Many potential causes of PRCA have been reported, but ~50% of cases have no known cause [6]. After epoetin had been used safely for many years without any significant immunogenicity, the incidence of epoetin-associated PRCA increased considerably from 1998 onwards [6,7], reaching a peak in 2002 [8]. Patients with epoetin-associated PRCA develop anti-erythropoietin antibodies, which neutralize any administered epoetin and also endogenous erythropoietin, leading to a depletion of erythroid precursor cells in the bone marrow [8].

The cases of epoetin-associated PRCA occurred mainly in patients receiving epoetin-α (EPREX®/ERYPO®). Ortho Biotech, a division of Janssen-Cilag; both products referred to by the term ‘EPREX®'.
hereafter) outside the USA, although a few patients received epoetin-β or a combination of products [9–11]. Irrespective of the type of epoetin administered, all but two of the cases (in patients with myelodysplasia [12]) occurred in CKD patients treated with subcutaneous (s.c.) epoetin; no cases have been confirmed in CKD patients treated only with intravenous (i.v.) epoetin or in cancer patients [11]. In view of these observations, a number of actions were taken to limit the increasing incidence of antibody-mediated PRCA attributed to EPREX® and to provide time to determine and eliminate the actual cause. These actions included a series of three ‘Dear Doctor’ letters to highlight the importance of the correct handling and storage of EPREX® (November 2001), to notify physicians that EPREX® should be administered i.v. where feasible (July 2002) and, finally, to notify physicians of a contraindication for s.c. administration of EPREX® in CKD patients in the European Union and Switzerland and a recommendation for i.v. administration elsewhere in the world (December 2002). At the same time, cold-chain management was improved by improving packaging and shipping methods, issuing revised guidelines on storage and handling, and developing educational materials [13].

Technical and clinical investigations were initiated to identify the cause of the increased immunogenicity of EPREX®. The technical investigation focused on the handling and storage of epoetin and on the chemical and biological characteristics of epoetin-α bulk drug substance and finished product. The clinical investigation focused on the patients, the route of administration and the type of epoetin product used. Potential causes of epoetin-associated PRCA were evaluated on the basis of several criteria. First, there should be a temporal relationship between the suspected cause and the increase in immunogenicity. The increase in incidence of epoetin-associated PRCA began in 1998, and so the potential cause should reflect a change that also occurred in or shortly before 1998. Secondly, epoetin-associated PRCA largely occurred in patients treated with EPREX®, and so any proposed cause would also have to reflect a significant difference between EPREX® and other epoetin products that appear to have a much lower incidence of PRCA. Thirdly, the strength of the suspected cause must be sufficient to elicit a weak immunogenic response. Confirmation of an increased immunogenic capacity by the suspected cause in an animal model would be supportive. Finally, any proposed cause must be consistent with the available clinical data.

In this article, we will describe the evidence that the appearance of organic compounds leaching from uncoated rubber stoppers of pre-filled syringes, due to the interaction with polysorbate 80 which was introduced as a new carrier solution of EPREX® in 1998, is the most likely cause of the increase in immunogenicity and fulfils all of the above-mentioned criteria. This evidence includes the observation that such compounds are not present in syringes with fluoro-resin-coated (FluroTec®) rubber stoppers or vials containing the polysorbate 80 formulation, in preparations containing human serum albumin (HSA), or in other epoetin products that have coated rubber stoppers [8]. In addition, experiments in mice demonstrated that the leachates could stimulate a dose-dependent immune response [8]. The only consistent difference between the end-products containing leachates and those not containing them was the use of coated rubber stoppers in the latter. Although it was standard practice to use uncoated rubber stoppers in 1994 when pre-filled syringes of epoetin were introduced, pharmaceutical companies have recently started using syringes with coated rubber stoppers, which provide a barrier between the stopper and the drug.

Technical investigation

PRCA is a very rare disorder [4], and even the greatly increased incidence of epoetin-associated PRCA from 1998 onwards only reached ~50 cases/100 000 patient-years during the peak years of 2001–2003 [14]. There are no published data that relate biopharmaceutical characteristics or excipients to immunogenicity at this very low incidence rate and, as a result, every possibility had to be explored. The technical investigation began with a review of the manufacturing data, and more sensitive analytical methods were developed to increase the number of product characteristics that could be monitored and to increase the sensitivity of existing tests. The product characteristics that were investigated included properties of the epoetin-α molecule itself, such as deamidation, methionine oxidation and aggregate formation in the finished product, and contamination with Chinese hamster ovary (CHO) protein from the host cells. In addition, components of formulated EPREX®, such as polysorbate 80, or its s.c. delivery system, such as silicone oil and other possible contaminants, were investigated (Table 1).

Structural changes and contaminants

Ten new analytical methods with increased sensitivity were developed to investigate possible changes in the structure of epoetin that may have altered its immunogenicity. The only change detected in bulk over the last 7.5 years was a very small increase in CHO protein, though this is still present in only nanogram quantities and constitutes <0.003% of total protein (Johnson & Johnson, data on file). No changes in deamidation, methionine oxidation or aggregate content were found between recent bulk and historical lots. Similarly, five new methods to analyse the finished product did not show any changes over time (Johnson & Johnson, data on file). There were no changes in primary structure or isoform distribution, and, although aggregate content and methionine oxidation increase slightly between syringe
release and expiry date, these do not rise above the allowed limits.

In mice, there was no clear evidence that CHO protein at concentrations of >100 times those present in EPREX/C213 formulations could induce an immune response to epoetin-α. Aggregates in concentrations of up to >50% or methionine oxidation >50% did not increase the immunogenicity in the same model (Johnson & Johnson, data on file).

Silicone oil

Silicone oil is used to lubricate all pre-filled syringes, including EPREX/C213, whether they are formulated with polysorbate 80, polysorbate 20 or HSA [8]. The amount of silicone oil expelled from pre-filled syringes of epoetin-α is variable and relatively high; however, it is the same for all EPREX/C213 syringes regardless of formulation and use of coated or uncoated rubber stoppers (Johnson & Johnson, data on file). The level of silicone oil expelled is highest for EPREX/C213, lowest for epoetin-β (NeoRecormon®, Roche), and intermediate for darbepoetin-α (Aranesp®, Amgen), thus there is no correlation between the level of silicone oil and the incidence of PRCA. The potential for silicones to act as adjuvants is still debatable. There is evidence in the literature that silicone gels can mediate immune reactions in experimental animals [15,16], though silicone gel does not appear to be immunogenic in itself [16]. The adjuvant capacity of silicone oils appears to be significantly lower than silicone gels and to depend on their molecular weight [17].

The siliconization process and type of silicone oil used in EPREX/C213 pre-filled syringes were defined in 1994, when the syringes were introduced, and have not been changed since then. There is thus no temporal correlation between the use of silicone oil and the increased incidence of epoetin-associated PRCA, and silicone oil did not increase the immune response to epoetin-α in mice. In addition, the same siliconization process has been used for the HSA-formulated syringes, the polysorbate 80 syringes with uncoated rubber stoppers, and the polysorbate 80 formulated product with coated rubber stoppers. Of these, only the polysorbate 80 formulated syringes with uncoated rubber stoppers demonstrated an increase in PRCA over baseline, indicating that silicone oil, by itself, is not the cause of PRCA.

Polysorbate 80

Polysorbate 80 was introduced as a stabilizer into the EPREX/C213 formulation in 1998, when HSA was removed because of concern over the possible transmission of infectious diseases. There is thus a temporal correlation with the increased incidence of epoetin-α associates with micelles of polysorbate 80, which may result in a structure that is sufficiently virus-like to be able to induce the development of anti-erythropoietin antibodies [22]. Epoetin-β did not appear to associate with micelles of polysorbate 20,

<table>
<thead>
<tr>
<th>Cause</th>
<th>Relevant a level in product</th>
<th>Level higher than in other epoetins</th>
<th>Change in 1998</th>
<th>Literature support for immunogenicity</th>
<th>Positive results from animal model</th>
<th>Evaluation summary b</th>
</tr>
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<td>No</td>
<td>Yes</td>
<td>No d</td>
<td>No</td>
<td>Unlikely</td>
</tr>
<tr>
<td>Oxidized methionine</td>
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<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Unlikely</td>
</tr>
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<td>Yes</td>
<td>No</td>
<td>Very unlikely</td>
</tr>
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<td>No</td>
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<td>No</td>
<td>Yes</td>
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<td>Very unlikely</td>
</tr>
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<td></td>
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<td></td>
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<td>Leachates</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Most likely</td>
</tr>
<tr>
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</tr>
<tr>
<td>Silicone oil</td>
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<td>No</td>
<td>No</td>
<td>Yes/No</td>
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<td>Unlikely</td>
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<td>Oxidized methionine</td>
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<td>No</td>
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<td>Unlikely</td>
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<td>Yes</td>
<td>No</td>
<td>Very unlikely</td>
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<tr>
<td>Deamidation</td>
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<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Very unlikely</td>
</tr>
</tbody>
</table>

aIndicates a level sufficient to cause an effect.
bBased on evaluation of data by Johnson & Johnson and a panel of immunology experts.
cLevels not different from those for epoetin-α manufactured in the USA.
dWe are not aware of any evidence that mammalian host cell protein has ever been the cause of increased immunogenicity in any therapeutic protein.

CHO = Chinese hamster ovary.
with which it is formulated. Investigations using light scattering, however, indicated that although epoetin-α associates with polysorbate 80, it does not form macromolecular structures with micelles of polysorbate 80 [8]. It was also shown that polysorbate 80 micelles are very unstable and dissipate within seconds after dilution [23]. Analytical ultracentrifugation has confirmed that neither polysorbate 20 nor polysorbate 80 form micellar-like structures coated with darbepoetin or epoetin-α [18].

In mice, polysorbate 80 in concentrations of up to 10 times that present in commercial formulations of epoetin-α did not increase the immunogenicity of epoetin-α [8]. In a comparative study of polysorbates 20 and 80, neither of these compounds over a wide range of concentrations increased the antibody response in mice primed with epoetin-α and incomplete Freund’s adjuvant (Johnson & Johnson, data on file). Thus, despite the temporal correlation with the appearance of epoetin-associated PRCA and the differences from formulations of other epoetin products, there is no evidence that polysorbate 80, by itself, increases the immunogenicity of epoetin-α.

Leachates from rubber stoppers

High-performance liquid chromatography (HPLC) has shown the presence of at least 10 non-peptide peaks in EPREX® formulated with polysorbate 80 delivered from syringes with uncoated rubber stoppers compared with coated rubber stoppers (Figure 1) [8]. Nine of the peaks have been identified and are potential forms or derivatives of a curing agent used in the making of the rubber stoppers [8]. The total amount of these compounds present in EPREX® increased with the storage time of the pre-filled syringes, increased with exposure to temperatures above 4°C, and was ~1–2 µg/syringe at 1–3 years storage [8]. It is known that polysorbates and other non-ionic detergents can leach material out of plastics [24], and it is most likely that the extra peaks are leachates from the uncoated rubber stoppers. In support of this, the leachates were not present in syringes with the same formulation but with fluoro-resin-coated stoppers (FluroTec®, Daikyo Seiko), in EPREX® formulated with HSA or in other epoetin products (Table 2). Sensitization to leachable plasticizers from synthetic rubber tubing used in medical equipment may predispose patients to hypersensitivity reactions during subsequent contact with synthetic rubber products [25].

In mice, anti-erythropoietin antibodies were not readily detected when the animals were injected with epoetin-α plus leachates from uncoated rubber stoppers stored at 5°C for 18 months: extra peaks

![Optimized, reversed-phase high-performance liquid chromatography profile comparing 18-month-old EPREX® formulated with polysorbate 80 from pre-filled syringes with uncoated rubber stoppers (upper curve) and coated rubber stoppers (lower curve). The contents of EPREX® syringes were injected into a Vydac C4 column. After a 5 min hold at 5% mobile phase B (0.06% trifluoroacetic acid in acetonitrile), samples were eluted at a flow rate of 1.0 ml/min by a linear gradient of 5–90% of mobile phase B for 90 min. Absorbance was measured at 280 nm. Reproduced from Sharma et al. with permission from the European Journal of Hospital Pharmacy [8]. EPO = erythropoietin.](http://ndt.oxfordjournals.org/)

Table 2. Relationship between the presence of leachates and the type of stabilizer and stopper

<table>
<thead>
<tr>
<th>Product</th>
<th>Polysorbate formulation</th>
<th>Rubber stoppers</th>
<th>Leachates present</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPREX® syringe</td>
<td>Yes (polysorbate 80)</td>
<td>Uncoateda</td>
<td>Yesb</td>
</tr>
<tr>
<td>EPREX® syringe</td>
<td>Yes (polysorbate 80)</td>
<td>Coated</td>
<td>No</td>
</tr>
<tr>
<td>EPREX® syringe</td>
<td>No</td>
<td>Uncoated</td>
<td>No</td>
</tr>
<tr>
<td>Darbepoetin syringe</td>
<td>Yes (polysorbate 80)</td>
<td>Coated</td>
<td>No</td>
</tr>
<tr>
<td>Epoetin-β syringe</td>
<td>Yes (polysorbate 20)</td>
<td>Coated</td>
<td>No</td>
</tr>
</tbody>
</table>

*Prior to April 2003; all stoppers are now coated.
*The presence of leachates depends on the age of these syringes; the amount increases with age.
HSA = human serum albumin.
stoppers [8], though such antibodies were elicited when the animals received epoetin-α plus incomplete Freund’s adjuvant. However, when ovalbumin was administered together with leachates, a dose-dependent immune response occurred (Figure 2) [8], indicating that the leachates have at least weak adjuvant activity. The lack of a detectable response with leachates and epoetin-α may have been due to the weaker adjuvant activity of leachates compared with incomplete Freund’s adjuvant and possibly interference from mouse erythropoietin in the antibody assay. Despite this, decreases in haematocrit in a number of mice injected with epoetin-α plus leachates were consistent with the production of anti-erythropoietin antibodies [8].

Although rubber stoppers were in use from the first introduction of EPREX® pre-filled syringes for s.c. administration, the replacement of HSA with polysorbate 80 in 1998 appears to have effected a change in the leaching of potentially immunogenic compounds from the uncoated rubber stoppers of EPREX® syringes. Epoetin-β and darbepoetin syringes for s.c. injection were introduced after those for epoetin-α and have coated stoppers. There is thus a difference from other epoetin products and a temporal association with the occurrence of epoetin-associated PRCA, which, together with the evidence of enhanced erythropoietin immunogenicity in the presence of leachates, strongly suggests that these compounds may have been an important contributory factor in the increased incidence of epoetin-associated PRCA.

Clinical investigation

To determine whether this hypothesis was consistent with the clinical data, reported cases of suspected epoetin-associated PRCA were analysed retrospectively by formulation of EPREX® used, dosage form and route of administration. This study was possible because from 2003, all dosage strengths of EPREX® for s.c. administration formulated with polysorbate 80 were packaged in pre-filled syringes with coated rubber stoppers, while, for some dosage strengths, this change had occurred in 2001.

Of the 217 patients with epoetin-associated PRCA who had anti-erythropoietin antibodies, 206 had received epoetin-α marketed as EPREX®/ERYPO® (of whom 23 had also received another epoetin) [14]. Among these patients, the vast majority (192 patients) had received s.c. EPREX®, while nine patients had received both i.v. and s.c. EPREX® and for five patients the route was unknown. None of the patients had received only i.v. EPREX®. On this basis, the rate of EPREX®-associated PRCA between January 1989 and June 2004 was 16.1/100 000 patient-years [95% confidence interval (CI), 14.0–18.5/100 000] for s.c. exposure (201 cases/1 244 970 patient-years) compared with 0/100 000 patient-years (95% CI, 0–0.4) for i.v. exposure (0 cases/871 098 patient-years) [14].

This analysis, however, includes many years when very few cases of epoetin-associated PRCA occurred, and it is more relevant to analyse data from the period when the occurrence of EPREX®-associated PRCA peaked. In this period, from 2001 to 2003, both types of syringes (those with uncoated rubber stoppers and those with coated rubber stoppers) were on the market simultaneously, allowing a comparison between the two. Over the years 2001–2003, a total of 146 cases of antibody-mediated PRCA occurred in CKD patients who had received only s.c. EPREX®, of whom 142 had received the polysorbate 80 formulation from pre-filled syringes with uncoated rubber stoppers (Table 3) [14]. The s.c. rate of EPREX®-associated PRCA among these patients was 46.1/100 000 patient-years of exposure, compared with 3.7/100 000 patient-years of exposure to all other EPREX® formulations/packages and 2.6/100 000 patient-years of exposure to the polysorbate 80 formulation with coated rubber stoppers. The rate difference between uncoated vs coated rubber stoppers with the polysorbate 80 formulation was highly significant (rate difference, 43.5/100 000 patient-years; 95% CI, 26.5–579), and the ratio between the rates was 17 (95% CI, 3.14–707).

In order to avoid bias, the analysis was then adjusted to account for exposure to multiple dosage forms. Cases with mixed exposure accounted for <20% of the total. Likewise, when cases with mixed exposure were excluded, estimates for the rate difference (35.0/100 000 patient-years; 95% CI, 26.5–43.6) and rate ratio (15; 95% CI, 2.6–579) between uncoated vs coated rubber stoppers with the polysorbate 80 formulation decreased by <20% when compared with non-adjusted estimates. Sensitivity analyses were done to adjust for changes in overall exposure due to the switch from uncoated to coated rubber stoppers for

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Fig. 2. Antibody response to ovalbumin and leachates. BDF-1 mice were injected s.c. on day 0 and day 14 with ~100 μg of ovalbumin with the addition of leachates at concentrations ranging from 0.3 to 2.7 μg/dose or with incomplete Freund’s adjuvant (IFA). Antibodies were measured by enzyme-linked immunosorbent assay on day 28. The amount of leachate is given as a multiple of the amount in one EPREX® syringe (×1). Reproduced from Sharma et al. with permission from the European Journal of Hospital Pharmacy [8].
pre-filled syringes. When the s.c. exposure to uncoated stopper syringes was decreased by 25%, based on an estimate in market shift, a substantial difference remained between the rate of EPREX\textsuperscript{a} with uncoated and coated syringe stoppers (38.9/100 000 patient-years; 95% CI, 29.2–48.6; \(P=0.0001\)), giving a rate ratio of 12 (95% CI, 2.2–448) [14].

These analyses show that EPREX\textsuperscript{a} almost exclusively occurred when EPREX\textsuperscript{a} formulated with polysorbate 80 was delivered from pre-filled syringes with uncoated rubber stoppers. Although pre-filled syringes with uncoated rubber stoppers had been introduced in 1994, leachates were only present after the change in stabilizer was made from HSA to polysorbate 80 in 1998. When the association between leachates and PRCA was first suspected in early 2003, the manufacture of all EPREX\textsuperscript{a} pre-filled syringes was switched from uncoated to coated rubber stoppers, and all pre-filled syringes with uncoated stoppers were subsequently recalled from pharmacies. This final risk-mitigation action produced a further decrease in the incidence of EPREX\textsuperscript{a}–associated PRCA, in addition to the recommendations to avoid the s.c. route and cold-chain improvements described earlier (Figure 3). There is some continuing s.c. use of the polysorbate 80 formulation of EPREX\textsuperscript{a} in pre-filled syringes with coated rubber stoppers that do not release leachates (20 000 patient-years in 2004). S.c. exposure to forms

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
EPREX\textsuperscript{a} form by s.c. route only & Case count & Exposure (patient-years) & Incidence/100 000 patient-years & 95% CI (Poisson exact) \\
\hline
Uncoated rubber stopper, polysorbate 80 formulation, pre-filled syringe & 142\textsuperscript{a} & 308 232 & 46.1 & 38.8–54.3 \\
Coated rubber stopper, polysorbate 80 formulation, pre-filled syringe & 1 & 38 608 & 2.6 & 0.07–144 \\
Human serum albumin formulation, all forms & 2 & 42 305 & 4.7 & 0.57–17.1 \\
Polysorbate 80 formulation, vials & 1 & 27 794 & 3.6 & 0.09–20.0 \\
Subtotal excluding uncoated rubber stopper, polysorbate 80 formulation, pre-filled syringe & 4 & 108 707 & 3.7 & 1.0–9.4 \\
\hline
Total & 146 & 416 940 & 35.0 & 29.6–41.2 \\
\hline
\end{tabular}
\caption{Case count, EPREX\textsuperscript{a} exposure and incidence of antibody-mediated PRCA in CKD patients with onset of loss of effect of epoetin during 2001–2003 and report received by 30 June 2004}
\end{table}
Epoetin-associated pure red cell aplasia

containing polysorbate 80 through April 2004 were included in the analysis, indicating that it is not associated with an increased incidence of PRCA [14]. The incidence of epoetin-associated PRCA is now back to almost baseline levels, but as the reported median time between first exposure to an epoetin and initial diagnosis of PRCA is 9.1 months (range, 0.3–82 months) [26], it can be anticipated that a few more cases of EPREX®-induced PRCA due to leachates may still occur. Once sufficient time has elapsed since the last exposure to leachates, the incidence of EPREX®-associated PRCA is expected to return to the baseline rate seen with all epoetin products.

Conclusions

The recommendation to administer EPREX® i.v., followed by the contraindication for s.c. use in the European Union and Switzerland, successfully reduced the incidence of EPREX®-associated PRCA. This did not address the cause of the increased immunogenicity of epoetin-α in EPREX®, but reduced high-risk exposure while investigations into the cause were ongoing. The technical investigation indicated that the most likely cause of the increased immunogenicity of epoetin-α that led to the increased incidence of EPREX®-associated PRCA was leachates extracted from the uncoated rubber stoppers of pre-filled syringes by the stabilizer polysorbate 80. No other changes in the epoetin-α molecule or the finished product outside normal ranges were identified. Leachates were shown to have a dose-dependent, weak adjuvant effect in animal models. Only the leachates fulfilled all of the criteria to be the causative factor for the increased incidence of anti-erythropoietin antibody-mediated PRCA.

The clinical investigation indicated that EPREX®-associated PRCA only occurred in patients treated with s.c. EPREX®, and that it was significantly associated with exposure to pre-filled syringes with uncoated rubber stoppers. While this is consistent with the leachate theory, the limitations of the case series analysis must be considered. These include the retrospective nature of the analysis and reliance on post-marketing surveillance data, in which information on dosages, formulations and presentations may be incomplete or inaccurate. The reporting of suspected EPREX®-associated PRCA could have been stimulated by the medical literature compared with the reporting of PRCA associated with other products. Inaccurate or low estimates of incidence may also result from use of historical data (pre-2001). However, all of the cases analysed in the clinical investigation reviewed here were classified by the same definition over the peak period of 2001–2003, when reporting was likely to be more complete. There is no reason to suspect that the reporting by EPREX® formulation is biased. The incidences determined in this clinical investigation are consistent with the results of a recent independent analysis [26].

In conclusion, the findings of the technical investigation, the animal data and the clinical investigation strongly suggest that leachates were the critical contributory factor in the increased incidence of antibody-mediated PRCA attributed to EPREX®.

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