Epoetin-Induced Autoimmune Pure Red Cell Aplasia

Nicole Casadevall,* Kai-Uwe Eckardt,† and Jérôme Rossert‡

*Department of Hematology, Hotel Dieu and INSERM U362, Paris, France; †Division of Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; and ‡Department of Nephrology, Tenon Hospital (AP-HP) and Pierre and Marie Curie University, Paris, France

During the first 10 yr of therapy with recombinant human erythropoietin (EPO), only three cases of antibody-associated pure red cell aplasia have been described in patients who were treated with EPO, whereas several millions of patients have received this treatment. Thus, the possibility for epoetin to induce the formation of anti-EPO antibodies was considered extremely low. However, since 1998, a significant increase in the number of cases of EPO-induced pure red cell aplasia has been found in patients with chronic kidney disease with a peak in 2001 and 2002. The incidence rate seems now to be back to the baseline level. The change in formulation of epoetin α sold outside the United States seems to be the cause of these antibodies.


Patients have been treated with recombinant human erythropoietin (EPO) since 1987/1988. EPO-induced antibodies remained a very rare complication for many years, with only a few case reports published (1–3). Since 1998, there has been an upsurge of cases of EPO-induced antibodies associated with pure red cell aplasia (PRCA) in patients who have kidney disease and receive subcutaneous treatment with EPO (4,5). To date, approximately 250 cases have been reported worldwide (6).

PRCA is an isolated disorder of erythropoiesis that leads to a progressively developing, severe, isolated anemia with sudden onset (7). Some factors are known to be associated with PRCA, but in approximately half of the cases, PRCA does not have an identifiable cause and is classified as idiopathic. The mechanisms that induce PRCA under these conditions have been shown to be mainly of autoimmune origin. A single case in which a patient who was never exposed to EPO developed PRCA as a result of autoantibodies against EPO has been reported (8).

Patients who have developed PRCA as a result of EPO antibodies have typically been on EPO therapy for 6 to 18 mo. The hemoglobin level then suddenly starts to decline, despite continued therapy with EPO at the same or even increased doses (4,5). The shortest time interval between start of EPO therapy and loss of efficacy observed in a single case was 2 mo, and the longest time interval was 90 mo.

Definite diagnosis of EPO antibody–induced PRCA requires two confirmatory investigations: A bone marrow examination and the demonstration of anti-EPO antibodies in patient serum. These investigations should be performed in each suspected case.

Analysis of the anti-EPO antibodies detected in 13 patients with EPO-induced PRCA has shown that they recognize the protein (4). The epitope that is recognized by the antibodies seems to be almost always conformational, and denaturation of the protein completely abolished the binding of the antibodies in all but one of these 13 cases. Because of its conformational nature, the precise mapping of the epitope has not yet been performed successfully. An analysis of 20 patients with EPO-induced PRCA showed that, in 18 cases, anti-EPO IgG were mostly IgG4, whereas in the two remaining cases, they were mainly IgG1 (S. Swanson, personal communication, 2004), which points to a role of T cells in the development of the antibody response.

The large majority of cases have occurred in patients who received EPO-α produced by Ortho-Biotech and marketed outside the United States (i.e., in patients who were treated with Eprex/Erypo) (6). Analysis of the data made available shows that between January 1998 and July 2003, 195 cases of antibody-mediated PRCA occurred in patients who were exposed to Eprex (subcutaneous route) either alone (177 cases) or in association with another EPO (18 cases), and that 63 cases are still being investigated. During approximately the same period of time, Roche, the manufacturer of EPO-β, reported 11 cases of antibody-associated PRCA in patients who had chronic kidney disease (CKD) and were exposed exclusively to EPO-β. Since July 1997, eight cases of EPO-induced PRCA have been observed with EPO-α produced by Amgen and marketed in the United States (i.e., Procrit, which is marketed by Ortho-Biotech, or Epogen, which is marketed by Amgen). Finally, Amgen has not reported any case in patients who were treated with darbepoetin α. Thus, the total number of cases of EPO-induced PRCA to date is probably close to 250.

Analysis of the annual incidence of EPO-induced PRCA per country shows very significant differences, even among different European countries; the European countries with the highest incidence of EPO-induced PRCA are France and the United Kingdom. The reasons for this difference are unclear. They cannot be explained by differences in market share between the different brands of EPO in these countries. It is possible that...
differences in storage and handling account for these differences in incidence of EPO-induced PRCA.

The total number of cases of EPO-induced PRCA shows that it progressively increased to peak in 2001 and 2002. Only one case was reported in 2003. No case was reported in the first half of 2004. Analysis of the route of administration of EPO in patients who developed PRCA shows that all patients were CKD patients who were receiving EPO subcutaneously.

In summary, the epidemiologic data indicate that subcutaneous administration of EPO is an important risk factor for the development PRCA but that additional factors associated with the use of the ex-U.S. formulation of EPO-α played an important role. So far, no case of EPO-induced PRCA has been reported in cancer patients on chemotherapy, although the anemia of malignant disease has become a frequent indication for EPO therapy. Only two cases have been observed in patients who were not treated for CKD-related anemia. Both were patients with myelodysplastic syndrome (9). Potential reasons for this apparent protection of cancer patients include shorter duration of therapy and unspecific immunosuppression.

Why This Upsurge in Antibodies Cases?

The observation that subcutaneous application of EPO is an important risk factor is in line with the fact that intravenous use of proteins is generally associated with the lowest risk for immunogenicity (10). Regarding the association with the ex-U.S. formulation of EPO-α, there is no reason to believe that the amino acid sequence of the molecule is different in this particular brand. Subtle differences exist between the carbohydrate moieties of EPO-α and -β (11,12), but whether they have any impact on immunogenicity remains unknown. Moreover, there is no indication that the glycosylation pattern of EPO-α has changed over time and could therefore explain the upsurge of cases.

The important clue apparently comes from the temporal coincidence with a change in the formulation of EPO-α to be sold outside the United States. Upon request from the European Agency for the Evaluation of Medicinal Products (London, UK) the manufacturers of EPO-α removed human serum albumin from the formulation and replaced it with polysorbate 80 to avoid potential contamination by viruses or prions. It has been postulated that this change in the formulation might have reduced the stability of the formulation, in particular when handling instructions, such as cold storage, were not followed. Contamination with silicone, used to lubricate prefilled syringes, has been considered as an additional risk factor. However, neither increased formation of aggregates has been demonstrated so far in vials of EPO-α.

Schellekens et al. (13) hypothesized an alternative explanation. They found that the concentration of polysorbate 80 in the new formulation of EPO-α is high and leads to micelle formation and that EPO molecules are integrated into the surface of these micelles. As a consequence, EPO molecules are presented to the recipient immune system in a regular spatial configuration, which could trigger the immune system.

It also remains possible that a contaminant present in the end product could act as an immunologic adjuvant. In fact, recent investigations focus on potential release of chemicals from the rubber stoppers of prefilled syringes. These rubber stoppers were used only for EPO-α syringes produced by Ortho-Biotech (Eprex/Erypo), and they have since been replaced by Teflon stoppers.

Response to Therapy

Obviously, once the diagnosis of EPO-induced PRCA is suspected or has been proved, EPO therapy needs to be discontinued. As far as investigated, anti-EPO antibodies cross-react not only with the endogenous hormone but also with all marketed recombinant EPO molecules. Therefore, increasing EPO doses or switching the brand does not improve the anemia and obscures the causality.

Available data suggest that cessation of EPO exposure alone is usually insufficient to induce recovery from EPO-induced PRCA. Retrospective analysis of 47 cases of EPO-induced PRCA (14) showed that 10 patients did not receive any specific treatment, besides stopping the administration of EPO. One patient died suddenly 6 wk after the diagnosis. All nine other patients still have PRCA after a median follow-up of 12 mo. In contrast, administration of immunosuppressive therapy seems largely to enhance the likelihood of recovery. Of 37 patients who received some immunosuppressive therapy, in addition to stopping EPO administration, anti-EPO antibodies disappeared and reticulocyte counts consistently rose above 20,000/mm³ in 78% of cases (n = 29). However, defining the optimal therapy is still somehow difficult. It is of note that in all cases with a regular follow-up of antibody titers, no increase in reticulocyte counts occurred as long as anti-EPO antibodies could be significantly detected by radio immunoprecipitation assay (RIPA).

Conclusions

Given that millions of patients are being treated with EPO, the prevalence of this complication remains very low, but the dynamics of the increase in incidence have initially caused great concern. Meanwhile, causes and risk factors have become somewhat clearer, and the incidence rates of EPO-induced PRCA seem to have passed the peak. Nevertheless, clinicians need to be aware of signs and consequences of this complication. Finally, this recent experience with anti-EPO antibodies may also have considerable implications for the future approval of EPO preparations and other biopharmaceuticals.

References
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