BRIEF COMMUNICATION

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Allergic Skin and Systemic Reactions in a Patient with Pure Red Cell Aplasia and Anti-Erythropoietin Antibodies Challenged with Different Epoetins

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Abstract. Recent concern has arisen about the development of neutralizing anti-erythropoietin (EPO) antibodies during the course of treatment with recombinant EPO. The underlying mechanisms are poorly understood. A patient was observed who developed wheals at the sites of subcutaneous injections of epoetin-α before the manifestation of pure red cell aplasia (PRCA). Intravenous application of different epoetins evoked skin reactions at the sites of former subcutaneous injections, indicating local persistence of sensitized cells, and eventually a systemic anaphylactoid response. Anti-EPO antibodies cross-reactive with epoetin-β and darbepoetin-α were demonstrated in patient serum. PRCA gradually improved after treatment with prednisolone.

Since recombinant human erythropoietin (EPO) became available in 1986, millions of patients have received the hormone for correction of renal and nonrenal anemias. EPO therapy is associated with few side effects, usually related to an increase in hemoglobin (Hgb) levels and the associated rise in blood viscosity. The extremely favorable risk/benefit relationship has in part been attributed to the close similarity between the endogenous and the recombinant hormone. The protein backbone of 165 amino acids is predicted by the human gene. The carbohydrate moiety consisting of three N-linked and one O-linked side chain closely resembles human urinary EPO, although slight differences exist between endogenous and recombinant EPO as well as between recombinant EPO preparations produced by different manufacturers (epoetin-α and epoetin-β) (1,2). Two additional sites for N-linked glycosylation have recently been introduced into the EPO gene to generate a recombinant epoetin molecule with increased stability, allowing less frequent applications (darbepoetin-α) (3).

All preparations of recombinant EPO have so far proven to be poorly immunogenic. During one decade, clinically relevant anti-EPO antibodies were observed in single cases only (4–7). In the last few years, however, there appears to be an increase in the number of patients developing neutralizing anti-EPO antibodies during the course of therapy (8,9). These patients develop aplastic pure red cell anemia, which responds poorly to immunosuppressive therapy and results in prolonged transfusion dependence. Although the total number of fewer than 100 cases reported so far is small in relation to the number of patients treated, this development raises some concern, because the true incidence is unknown and the underlying mechanisms remain unclear. Interestingly, all 13 cases of patients with anti-EPO antibodies for which details have recently been published (8) have received epoetin therapy via the subcutaneous route. We report a patient with anti-EPO antibodies in whom intravenous injection of epoetins has evoked skin reactions at sites of former subcutaneous injections, suggesting that immune reactions in the subcutis play an important role in antibody generation.

Case Report

A 48-yr-old male patient with renal failure due to suspected chronic glomerulonephritis and no history of allergy commenced therapy with epoetin-α (3×2000 IU/wk subcutaneously) in August 2000 (Figure 1). Within 10 wk, his Hgb rose from 10.6 g/dl to 13 g/dl. Nine months later, he was started on regular three-times-a-week hemodialysis because of further decline in his renal function. Epoetin-α was continued at doses of 1 to 3×2000 IU/wk. From the beginning of June 2001, the patient noticed single wheals at the injection sites of subcutaneous epoetin on the abdomen, which always disappeared within 8 to 10 h after the injection. Occasionally epoetin-α was injected intravenously and then weak wheals appeared at sites of former subcutaneous injections. Eight weeks after these skin reactions were first observed, the hematocrit dropped to 24%.

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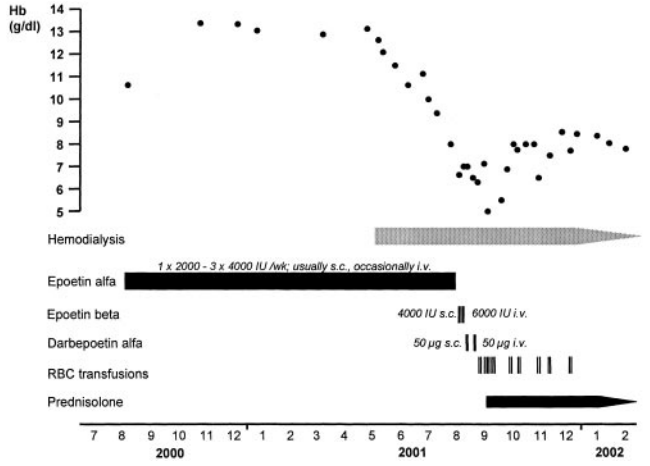

Figure 1. Clinical course of a patient developing pure red cell aplasia during treatment with epoetin (EPO). First skin reactions at the sites of subcutaneous epoetin injections occurred in June 2001.

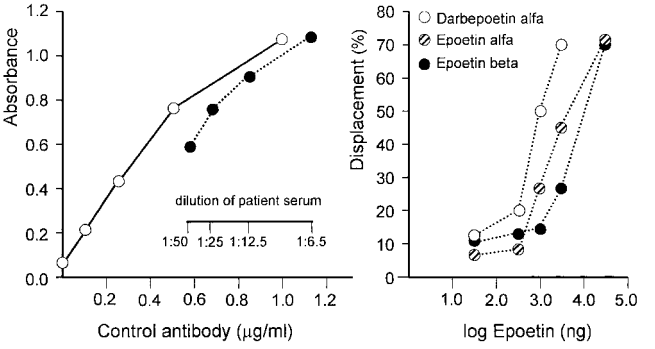

Figure 2. Demonstration of anti-EPO antibodies in patient serum. The antibody assay used is a sandwich enzyme-linked immunosorbent assay (ELISA), performed in microtiter plates pre-coated with epoetin-β. Anti-EPO antibodies binding to immobilized epoetin are detected with anti-human IgG conjugated to horseradish peroxidase. The control antibody is a conjugate of polyclonal rabbit anti-human EPO antibody and human IgG. Left panel shows parallel dilution curves for control antibody (○) and patient serum (●) obtained September 2001. To differentiate between nonspecific and specific binding, a competitive displacement reaction step using 100 μg of epoetin-β is routinely performed for each sample. In all samples of the patient tested during the observation period, displacement was >70%. The right panel illustrates displacement curves with different epoetins using patient serum obtained September 2001 (1:25 dilution), indicating crossreactivity of the antibodies with epoetin-α, epoetin-β and darbepoetin-α.

The epoetin-α dose was increased to 3 × 4000 IU subcutaneously for 2 wk. There were no clinical or laboratory signs of bleeding, iron deficiency, inflammation, hemolysis, vitamin B12, or folate deficiency, aluminum overload, or severe hyperparathyroidism. In August 2001, the patient was switched to epoetin-β because skin reactions were assumed to be due to some constituent of the epoetin-α formulation. He received two injections of 4000 and 6000 IU epoetin-β, one subcutaneously and one intravenously, and both resulted in skin reactions similar to those previously observed. Epoetin-β was then replaced by 50 μg of darbepoetin-α subcutaneously 1 wk later. Hours later, localized erythema and burning, which resolved until the next day, occurred at the injection site on the upper thigh. One week later, a second dose of darbepoetin (50 μg) was given intravenously. Within seconds, the patient experienced a short period of generalized heat sensation, which was followed a few minutes later by a pronounced urticaria with red, confluent wheals at the sites of previous injections of epoetin-α and -β on his abdomen. This reaction was more intense than those that followed previous intravenous applications of epoetin-α or epoetin-β. Methylprednisolone was administered intravenously, and urticaria and itching improved substantially within minutes. Epoetin treatment was then discontinued.

In the course of the subsequent hospitalization, the patient's Hgb was determined at 6.4 g/dl with no reticulocytes detectable on a blood smear and red blood cell transfusions were given. Bone marrow aspiration and biopsy were performed, and the diagnosis of pure red cell aplasia was made. The bone marrow aspiration was unfortunately complicated by a gross retroperitoneal hematoma and a lesion of the lumbosacral plexus. During the course of further work-up, anti-EPO antibodies were found in serum samples using two independently developed enzyme-linked immunosorbent assays (ELISA) in two different laboratories. Replacement curves showed binding activity of the patient serum to epoetin-α, epoetin-β, and darbepoetin-α (Figure 2). The level of endogenous serum EPO was not significantly above the detection limit of the assay (EPO ELISA, R & D Systems, Wiesbaden, Germany).

During 18 wk, a total number of 18 red blood cell transfusions were required to maintain an Hgb value of 7 to 8 g/dl. A treatment course with prednisolone was begun in September 2001 (1 mg/kg daily orally for 4 wk, with subsequent tapering of the dose). During subsequent weeks, anti-EPO binding activity in patient serum declined to levels roughly corresponding to 20% of the binding activity observed in September 2001. Fourteen weeks after the onset of steroid therapy, the patient started to maintain a Hgb concentration of approximately 8 g/dl without further transfusions, and his free serum EPO level had increased to 15.7 mU/ml (reference range, 3.3 to 16 mU/ml). He was successfully transplanted 3 mo later.

Discussion

Subcutaneous injection of epoetin is the preferred treatment route in patients not on hemodialysis (10,11). In patients on hemodialysis, thrice weekly subcutaneous injection has been recommended because it is considered to be more efficient than thrice weekly intravenous injection (11). The bioavailability of epoetin, as determined by the area under the curve of plasma hormone levels is, however, much lower after subcutaneous than after intravenous injection (12). This suggests that reabsorption from subcutaneous tissue is incomplete, but the fate of epoetin injected subcutaneously has so far not been addressed. Although the time intervals between subcutaneous application and secondary skin reactions evoked at previous injection sites by intravenous administration of epoetin were not precisely

determined in the present case, the overall extent of localized wheals suggests that sensitized immune cells persisted for several weeks. Skin has a highly developed immune system with different cell types contributing to antigen presentation and immune responses (13). It is therefore tempting to speculate that prolonged exposure of these cells to epoetin after subcutaneous administration facilitates the generation of anti-EPO antibodies.

Additional factors must be postulated, however, which have led to an increase in immunogenicity of injected epoetin in recent years. The majority of patients developing anti-EPO antibodies, including the present case, generated antibodies in response to epoetin-α distributed outside the United States (8,14). Anti-EPO antibodies were found to be directed against conformational epitopes of the protein moiety of the molecule (8), but an alteration of the amino acid sequence of the recombinant product is highly unlikely. It is conceivable that slight differences in the glycosylation pattern or secondary changes induced by formulation or storage conditions lead to the presentation of previously hidden epitopes or generation of structures that are immunogenic. Anti-EPO antibodies developed in this way are crossreactive with the endogenous hormone, leading to more severe anemia than in the absence of EPO therapy. In addition, anti-EPO antibodies in the present and in 13 additional cases reported also crossreacted with different epoetins, including darbepoetin-α (8). A recommendation has meanwhile been issued to discontinue therapy with recombinant erythropoietic products rather than switch patients to other preparations in cases of treatment failure (9). The symptoms observed in the present case after treatment attempts with different epoetins, which were conducted in unawareness of the underlying mechanisms, strongly support this advice.

In conclusion, this case provides evidence for an important role of the subcutaneous route of administration of epoetin in the generation of antibodies and shows that skin reactions at the injection site may be a first sign of sensitization, which can precede the development of severe anemia by several weeks. Moreover, we document for the first time that continuation of epoetin therapy in patients with anti-EPO antibodies carries the danger of systemic anaphylactoid reactions.

Note Added in Proof

Since submission of this article, information about epoetin-associated cases of PRCA reported to the FDA and an update of the number of cases studies by Casadevall *et al.* have been published (15,16).

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