Serum Erythropoietin Levels in Patients Receiving Intensive Chemotherapy and Radiotherapy


To investigate the potential role of recombinant human erythropoietin (rhEpo) in patients receiving intensive cytotoxic therapy, we measured the endogenous levels of Epo in 31 patients undergoing intensive marrow transplantation (BMT). Seventeen patients underwent allogeneic BMT and 14 underwent autologous BMT. On average, 10 ± 4 units of red blood cells (RBCs) were transfused per patient. The mean RBC transfusion requirement of the autologous BMT patients was significantly greater than that of the allogeneic recipients (12 ± 3 vs 8 ± 4, P < .01), although both groups were maintained at comparable hematocrits. Epo levels were measured by radioimmunoassay (RIA). For each patient, baseline serum Epo levels were determined at the time of admission to the hospital. Subsequent samples were collected within 24 hours of completing chemotheraphy and/or radiotherapy, and on days 7, 14, and 28 after BMT. Hematocrits (Hcts) were measured daily. All patients had an initial serum creatinine ≤ 1.5 mg/dl. Despite considerable differences in absolute Epo levels among individuals, a characteristic pattern was observed. Following admission to the hospital and initiation of cytotoxic therapy, the average Hct decreased and the average Epo level initially increased. The mean serum Epo levels peaked on day 7 post-BMT (284 ± 190 mU/ml) and fell steadily thereafter. While the average Hcts on day 7 and on day 28 post-BMT were not significantly different (28 ± 4.6% vs 29 ± 3.3%, respectively), the average serum Epo levels decreased fourfold (P < .01) during this same period. Moreover, day 28 post-BMT mean Epo levels were inappropriately low (P < .05) when compared with a reference population with bone marrow failure and normal controls who had not received cytotoxic therapy. We conclude that the endogenous Epo response appears to be blunted during the 3 to 4 weeks immediately post-BMT. Therefore, clinical trials assessing the efficacy of the administration of rhEpo in the treatment of anemias associated with cytotoxic therapy are warranted.

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MATERIALS AND METHODS

This study was approved by the Institutional Review Boards of each of the participating institutions and informed consent was obtained from each study participant before beginning the study. Patient population. Patients entering the Brigham and Women’s Hospital, Dana-Farber Cancer Institute, and the Beth Israel Hospital (Boston, MA) for either autologous or allogeneic BMT were enrolled in the study. Serum samples were collected on the first hospital day (before initiation of cytotoxic therapy), within 24 hours of completing cytotoxic therapy (hereafter designated day 0), and on days 7, 14, and 28 after BMT. Preparative regimens varied according to the protocols used by each participating institution and the patient’s diagnosis (Table 1). Patients had complete blood counts monitored daily and electrolytes, renal function tests (serum blood urea nitrogen and creatinine), and liver function tests monitored at least three times weekly. In general, patients received RBC transfusions to maintain a hematocrit (Hct) in the vicinity of 25%.
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<th>Patient Characteristics</th>
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<td><strong>Abbreviations:</strong> ADR, adriamycin; ARA-C, cytosine arabinoside; BLE, bleomycin; Carboplatin, carboplatin; CDDP, cisplatin; CHL, chlorambucil; CTX, cyclophosphamide; DAT, DAUNO, ARA-C, 6-TG; DAUNO, ARA-C, mitoxantrone; DBP, daunorubicin; EPO, epoetin; EPO, epoetin alfa; MDS, myelodysplastic syndrome; MM, multiple myeloma; RA; refractory anemia; RBC, red blood cells; TBI, total body irradiation; 6-TG, 6-thioguanine; THIO, thiotepa; TX, transfusion;</td>
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Reference population. Eleven volunteers with normal Hcts and normal renal function served as controls. Sixteen patients with well-documented aplastic anemia or myelodysplastic anemia who had normal renal function and had never received chemotherapy served as a comparison group. Together these 27 subjects comprised the reference population.

Statistical methods. Of the 31 patients completing the study, one patient (no. 4) was eliminated from the analysis. This patient suffered an acute massive gastrointestinal hemorrhage during the fourth week of hospitalization that produced a marked increase in Epo level and transfusion requirement.

Therefore, the data base consisted of: (1) Hct and serum Epo measurements made on each of 30 BMT patients at the five time intervals noted above; and (2) Hct and Epo measurements made at a single point in time on the 27 subjects in the reference population. The Hcts tended to be normally distributed, and therefore were correctly represented by the mean ± 1 SD, and analyzed by parametric methods. The Epo distributions were log-normally distributed and better represented by the geometric mean, particularly in regression analysis. However, for consistency of reporting and discussion, and because outcome is not affected by use of the less efficient central measure, the mean ± 1 SD was used for all distributions. Comparison of the Hct and Epo data between the respective time periods and the reference population was done using appropriate two sample and multiple sample comparison tests.

Epo radioimmunoassay (RIA). Serum immunoreactive Epo was measured by a method similar to that described by Egrie et al.20 The RIA for Epo was performed using a high-titer polyclonal rabbit anti-rhEpo serum produced in our laboratory. 125I rhEpo was obtained from Amersham, Inc (Arlington Heights, IL). Standards were prepared using rhEpo from Amgen, Inc (Thousand Oaks, CA) diluted in minimal essential medium Alpha containing 0.5% bovine serum albumin (BSA) and 0.05% sodium azide, pH 7.4. Aliquots of 0.2 mL of standard or sample were placed in 5-mL conical polypropylene tubes. To this was added 0.1 mL of rabbit antiseraum diluted 1:15,000 in phosphate-buffered saline (PBS) containing 0.5% BSA. The mixture was diluted to 0.7 mL using the same diluent used for the recombinant Epo standards and was incubated at room temperature for 2 hours. 125I Epo, 0.1 mL (~15,000 cpm), prepared in the same diluent was then added, the mixture was briefly vortexed, and incubated for 12 to 16 hours at 4°C. Tachisorb R Immunosorbent (goat antirabbit γ-globulin conjugated Pansorbin Staphylococcus aureus Cells; Calbiochem), 0.6 mL, was then added to each tube and the tubes were placed at 4°C with constant shaking for 3 hours. The Tachisorb was pelleted by centrifugation for 30 minutes at 1,500g at 4°C, washed once with 2.0 mL PBS, and counted in an LKB model 2282 gamma counter. Assays on patient serum were performed in duplicate and at two different dilutions. Using this assay, the normal range of serum Epo levels is 16 to 38 mU/mL calibrated against the World Health Organization Second International Reference Standard.

RESULTS

Patient characteristics. Of the 30 patients included in the analysis, 17 underwent allogeneic BMT and 13 received autologous BMT. Patient characteristics, including age, sex, diagnosis, type of transplant, previous chemotherapy and radiation therapy, conditioning regimen, transfusion requirements, Hcts, and Epo levels are presented in Table 1. Because hematocrit values were maintained artificially by RBC transfusions, the patients are presented in Table 1 in order of decreasing numbers of transfusions. Of note, one patient (no. 22) developed renal failure (serum creatinine > 1.5 mg/dL) beginning on day 11 posttransplant with a peak creatinine of 2.2 mg/dL on day 27. All the other patients had serum creatinine values ≤1.5 mg/mL throughout the period of study.

Epo levels as a function of time and Hct. The Epo levels and Hcts are summarized in Table 2 and Fig 1. Mean Epo levels initially increased after patients received cytotoxic therapy from 79 ± 133 mU/mL on the day of admission to a peak value of 284 ± 190 mU/mL measured on day 7 post-BMT. As might be expected, this initial increase in mean serum Epo levels between the day of admission and day 7 corresponded to a significant decrease in mean Hct (32 ± 4.7% v 28 ± 4.6%, respectively; P < .01). However, while the mean Hcts were essentially constant on days 7, 14, and 28 post-BMT (28% ± 6.4%, 28% ± 3.1% and 29% ± 3.3%, respectively), the mean serum Epo levels decreased almost fourfold during this same period (284 ± 190 mU/mL, 165 ± 135 mU/mL, and 75 ± 48 mU/mL respectively; P < .01), a behavior that is not consistent with expectations. This suggested a decreased Epo response to anemia between approximately day 14 and day 28 post-BMT.

To further evaluate the impact of cytotoxic therapy on serum Epo levels as a function of Hct, the mean In Epo levels and corresponding Hcts for the five time points were plotted together with the In Epo levels and Hcts of the reference population. This enabled us to compare two groups of patients with comparable degrees of bone marrow failure and study the impact of cytotoxic therapy on serum Epo levels. Figure 2 shows that the reference In Epo levels increase with the degree of anemia. The mean In Epo levels obtained from the transplant patients administered cytotoxic therapy fell below the line generated by the reference population and outside the 95% confidence intervals. The admission and day 14 post-BMT mean In Epo levels are 1.2 SD below the regression line (NS), while day 0 and day 7 post BMT levels

Table 2. Mean Hcts and Epo Levels

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<td>Admission</td>
<td>32 ± 4.7</td>
<td>79 ± 113</td>
<td>31 ± 5.3</td>
<td>56 ± 38</td>
<td>33 ± 4.2</td>
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<td>Day 0</td>
<td>28 ± 3.9</td>
<td>213 ± 141</td>
<td>28 ± 4.1</td>
<td>239 ± 129</td>
<td>29 ± 3.9</td>
<td>194 ± 149</td>
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<td>Day 7</td>
<td>28 ± 4.6</td>
<td>284 ± 190</td>
<td>26 ± 2.5</td>
<td>329 ± 188</td>
<td>28 ± 5.7</td>
<td>251 ± 189</td>
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<td>Day 14</td>
<td>28 ± 3.1</td>
<td>165 ± 136</td>
<td>27 ± 2.9</td>
<td>194 ± 164</td>
<td>28 ± 3.4</td>
<td>144 ± 112</td>
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<td>Day 28</td>
<td>29 ± 3.3</td>
<td>75 ± 48</td>
<td>30 ± 2.1</td>
<td>64 ± 46</td>
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RBC units: 10 ± 4
EPO LEVELS WITH CYTOTOXIC THERAPY

Twenty of the 30 patients received amphotericin B at some point during the 28 days post-BMT. Although amphotericin B has been reported to inhibit Epo production, the typical pattern of increase and subsequent decrease in serum Epo levels did not appear to correlate with amphotericin B administration (patient nos. 3, 5, 9, 15, 17, 25, and 28 through 31 did not receive amphotericin B). Similarly, although an increase in serum Epo levels has been noted when patients with renal insufficiency develop hepatitis, review of the liver function tests in our patient population failed to show an association between changes in liver function and serum Epo levels.

Transfusion requirements. As a group, patients who received autologous transplants had a significantly higher transfusion requirement than those receiving allogeneic transplants (12 ± 3 vs 8 ± 4, P < .01; Table 2) even though they were maintained at comparable Hcts at each point in time.

**DISCUSSION**

We have prospectively measured endogenous serum immuno reactive Epo levels in 31 patients receiving intensive chemotherapy and combination chemotherapy and radiation therapy in preparation for autologous or allogeneic BMT. With the current availability of accurate immunoassays and the potential to intervene with rhEpo, investigators have begun to survey anemias of diverse etiologies to determine if the Epo response is appropriate. One difficulty in this approach is determining what is an appropriate reference population with which to compare the adequacy of the Epo response. For patients receiving myelosuppressive and ablative doses of chemotherapy and radiotherapy, it seems appropriate to compare their serum Epo levels to patients with bone marrow failure (aplastic and myelodysplastic anemias) who have not been exposed to cytotoxic therapy. Using this approach, it becomes quite evident that by 4 weeks after cytotoxic therapy many of these patients have a markedly decreased serum Epo level for their degree of anemia (Fig 2). In addition, compared with their own previous Epo response to a given degree of anemia from admission through day 7 post-BMT, these patients appeared to develop a decreased Epo response to a given degree of anemia (Fig 1 and Table 2).

Similar to several previous studies, we noted a characteristic pattern of increasing serum Epo concentrations after cytotoxic therapy. These previous reports suggested that the Epo increase was caused by a mechanism other than increasing anemia and triggering of the oxygen sensor in the kidney. By comparing the data with our reference population we found that the observed decrease in the Hct during this period appeared to be enough to explain the increase in Epo levels. However, within the study group it must be noted that there did appear to be a small but statistically significant increase in mean serum Epo levels between day 0 and day 7 post-BMT (213 ± 140 mU/mL v 284 ± 184 mU/mL; P < .05), which occurred without a significant change in Hct (28% ± 3.9% v 28% ± 4.6%, NS). This may reflect a greater degree of marrow aplasia present by day 7 post-BMT because it has been suggested that patients with marrow aplasia may have an exaggerated Epo response for a given
degree of anemia. Other possible explanations include cytotoxic therapy (1) causing direct injury to the Epo-producing cells in a manner that mimics hypoxia; (2) altering blood flow to the kidney and/or liver in such a way as to expose the Epo-producing cells in these organs to an increased degree of hypoxia; or (3) disrupting the usual Epo degradation pathway(s) and thereby extending the serum half-life of the protein. In addition, chemotherapy and radiation therapy may clearly interfere with ongoing protein synthesis and gene transcription. In view of the finding that ongoing protein synthesis and gene transcription are necessary for the normal degradation of Epo messenger RNA (mRNA), it is possible that this increase in serum Epo levels may be a reflection of enhanced Epo mRNA stability following such cytotoxic therapy.

Patients undergoing autologous BMT were unexpectedly found to require significantly more RBC transfusions in the first month post-BMT than the group of patients receiving allogeneic BMT (although they had comparable Hcts at each point in time studied). One possible explanation for this may be that the autologous bone marrow progenitor and stem cells were already somewhat damaged by previous treatment with cytotoxic therapy (Table 1). This finding may also be related to differences in the conditioning regimens between the autologous and allogeneic BMT patients (Table 1).

The etiology of the decreased Epo response to anemia that these patients demonstrated remains open to speculation. The onset of the blunted response occurring about 2 weeks after cytotoxic therapy is similar in timing to the myelosuppression after such therapy. Although the exact nature of the Epo-producing cells in the kidney and liver is controversial, it is possible that the Epo-producing cells are damaged by the chemotherapy and radiotherapy. This has been suggested as the etiology of the anemia associated with cisplatin chemotherapy, although direct erythroid marrow toxicity may also play a role. Because most of these patients are exposed to multiple potential nephrotoxic drugs during their hospital course, one cannot completely exclude the potential contributions of these agents to renal dysfunction. Serum creatinine levels were stable in all but one patient, although the serum creatinine is a fairly crude measure of glomerular filtration rate. Furthermore, almost all patients develop fevers at some point in the month after BMT. It is possible that this blunted Epo response is related to the anemia of chronic disease. Some studies have suggested that the anemia of chronic disease is associated with a blunted Epo response, while other studies failed to show any association.

Although the Epo response to anemia is blunted, analogous to the situation with chronic renal failure, these patients still seem able to respond to hypoxia with an increase in serum Epo levels. This is demonstrated by patient no. 4 who was able to markedly elevate his serum Epo level in response to a severe anemia brought on by an acute, large upper gastrointestinal hemorrhage. Nonetheless, this markedly decreased Epo response contributes substantially to the pathogenesis of the anemia of renal failure and, in a similar fashion, may contribute to the anemia following cytotoxic therapy.

While the etiology is open to speculation, it is clear that during the first 4 weeks after BMT, these patients treated with large doses of cytotoxic therapy manifested not only a decreased Epo response for a given degree of anemia compared with their own day 7 post-BMT response, but they also showed a decreased response compared with the reference population. Thus, clinical trials assessing the efficacy of rhEpo in the treatment of anemias associated with intensive cytotoxic therapy appear warranted.

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