A Randomized Study of Erythropoietin and Granulocyte Colony-Stimulating Factor (G-CSF) Versus Placebo and G-CSF for Patients With Hodgkin’s and Non-Hodgkin’s Lymphoma Undergoing Autologous Bone Marrow Transplantation

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Anemia is a universal finding in patients undergoing autologous bone marrow transplantation (BMT). Effective therapies to increase the number of autologous red blood cells could result in a lower morbidity and mortality associated with red blood cell transfusions. We examined whether the addition of erythropoietin (Epo) to intensive therapy supported by progenitor cell transplantation and granulocyte colony-stimulating factor (G-CSF) would result in a lower requirement for red blood cell transfusions. Thirty-five patients with lymphoma were randomized to receive Epo versus placebo. Epo (600 U/kg three times per week) or placebo was begun 3 weeks before administration of high-dose therapy. Epo was held during the week of the preparatory regimen, and restarted on the day after BMT. All patients also received G-CSF following BMT. No significant differences were noted between the two groups in terms of patient characteristics at pretreatment or post-BMT evaluation. There were no differences in the total number of red blood cells transfused (median Epo: 8 v placebo: 6, P = .22) nor the number of platelet transfusions given (median Epo: 12 v placebo 5, P = .14). Engraftment of granulocytes (absolute neutrophil count ≥500/μL) occurred in a median of 12 days (range, 9 to 33) for the patients receiving Epo and G-CSF, compared with a median of 10 days (range, 8 to 22) for those receiving placebo and G-CSF (P = .22). Likewise, there were no differences in the time to platelet count ≥20,000/μL without further transfusions with a median of 22 days (range, 15 to 150+) for those receiving Epo and G-CSF compared with a median of 20 days (range, 11 to 54) for those patients receiving placebo and G-CSF (P = .28). The combination of G-CSF and Epo as administered in this study appears to be safe but does not result in an improvement in the total number of red blood cell transfusions or total number of single donor platelet units transfused.

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THE SUCCESSFUL USE of bone marrow transplantation (BMT) in the treatment of a variety of neoplastic diseases has brought into focus the need to minimize transplantation-associated morbidity. Profound suppression of all three cell lineages is uniformly observed in patients undergoing BMT. Supportive measures such as protective isolation and myeloid growth factors are used until a functioning myeloid graft is established. The use of granulocyte-macrophage colony-stimulating factor (GM-CSF) or granulocyte colony-stimulating factor (G-CSF) is associated with accelerated myeloid engraftment. Anemia and thrombocytopenia can be treated by transfusion therapy, however, significant complications such as febrile reactions, viral infections, and alloimmunization commonly occur. It seems likely that therapies directed at reducing the duration of marrow aplasia of all three cell lines would decrease the morbidity and mortality associated with infection and bleeding in the immediate posttransplantation period.

Several clinical trials have demonstrated the ability of erythropoietin (Epo) to reverse the anemia associated with chronic renal failure. A dose dependent increase in both the hematocrit and the reticulocyte count is observed with doses ranging from 15 to 500 U/kg administered 3 times per week. Epo was well tolerated with exacerbation of pre-existing hypertension being the only significant side effect. In the setting of multiple myeloma, Epo therapy resulted in a normalization of anemia and an improved performance status. This was accompanied by an increase in erythroid burst-forming units in the bone marrow as well as in the peripheral blood. Epo has also been given to cancer patients as therapy for the anemia of non-myeloid malignancies and in 1993 was licensed by the FDA for this indication.

As the biologic activity of a variety of hematopoietic growth factors becomes better understood, evaluation of combinations of factors to accelerate the engraftment of all three cell lines following BMT is warranted. The successful development of such combination therapies requires that the activity of each individual growth factor be defined and that careful studies of combinations of growth factors be undertaken to determine whether enhanced efficacy might be achieved. In this study, we examined the effects of Epo and G-CSF compared with placebo and G-CSF on hematopoietic engraftment in patients undergoing autologous BMT.

PATIENTS AND METHODS

Informed consent. All clinical protocols were approved by the Institutional Review Board at Stanford University Medical Center. The risks and benefits were explained to each patient in detail during his/her out-patient visits and again on the day of enrollment, and a written consent was obtained.

Patients. From February 1992 through July 1993, patients with relapsed Hodgkin’s and non-Hodgkin’s lymphoma undergoing autologous BMT were eligible for this study. Patients with Hodgkin’s disease were transplanted with peripheral blood progenitor cells (PBPC) and bone marrow. Patients with non-Hodgkin’s lymphoma received monoclonal antibody and complement purged bone marrow. Nearly all patients had received at least two different combination chemotherapy regimens before initiation of BMT. All but two patients responded to cytoreductive chemotherapy before BMT. The patient characteristics were well balanced between these two groups.

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Growth factor administration. Epo (epogen) was supplied by Ortho Biotech (Raritan, NJ). G-CSF (filgrastim) was produced by Amgen Corporation (Thousand Oaks, CA). Both factors were supplied through the Cancer Therapy Evaluation Program of the National Cancer Institute. Epo or placebo was begun 3 weeks before admission for BMT (Fig 1), on the day after bone marrow harvesting. The Epo dose was 600 U/kg administered three times per week intravenously through a central catheter over several minutes. The rationale for intravenous administration was that the volumes (up to 6 mL) were too high for subcutaneous administration. Epo was then held during the administration of the myeloablative regimen (day −7) and restarted 1 day after infusion of the bone marrow (day +1) and continued three times weekly until day +30 or until the hematocrit rose to 46%. Packed red blood cell transfusions were given to patients on day +1 and continued until the patient had three consecutive days of ≥500 granulocytes/μL. In patients undergoing collection of mobilized PBPC, G-CSF (Amgen) was administered at a concentration of 10 μg/kg/d, administered intravenously or subcutaneously 4 days before initiation of apheresis and continued daily until the PBPC collection was completed. If the white blood cell (WBC) count exceeded 60,000/μL, G-CSF was held until the WBC fell below 60,000/μL. Collection of these PBPC occurred after the bone marrow harvest and before administration of Epo. Three patients with Hodgkin’s disease also received cyclophosphamide (4 g/m²) for cytoreduction and PBPC were harvested at the time of WBC recovery (2, placebo; 1, Epo). These three patients received Epo while undergoing apheresis.

Apheresis and storage. A 12 French double lumen Hickman type catheter (Cook, Bloomington, IN) was placed before apheresis. Apheresis was performed using a Cobe Spectra (Cobe, Denver, CO), and 10 to 14 L of whole blood was processed during each collection procedure. Platelet counts were maintained above 60,000/μL at all times with irradiated platelet concentrates (2,500 cGy). Apheresis was continued daily until the target cell dose of 10⁹ mononuclear cells/kg was collected. Bone marrow was collected as previously described. The bone marrow and PBPC were frozen in a controlled-rate freezer (Cryomed) at −1°C per minute to a temperature of −50°C and at −10°C per minute to a temperature of −70°C and then transferred to the liquid phase of a liquid nitrogen freezer.

Colony assays and flow cytometry. Aliquots (1 mL) of the collections from each patient were thawed and pooled. Mononuclear cells were suspended in Iscove modified Dulbecco medium with 20% fetal calf serum, 50 mmol 2-β mercaptoethanol in 1.1% methylcellulose for viscous support, using 17% human placental-conditioned medium as a source of colony stimulating factor, as previously described. The mononuclear cells were plated at a final concentration of 4 × 10⁴ cells/mL in Costar Mark II tissue culture plates (24 to 16 mm diameter microtitre wells, Costar Corp, Cambridge, MA) in a final volume of 0.25 mL. Myeloid and erythroid colonies were counted after 10 days of incubation at 37°C in a humidified incubator containing room air with 5% CO₂.

Cells expressing the surface marker CD 34, were identified by indirect immunofluorescence with the monoclonal antibody HPCA-1 (Becton Dickinson, Mountain View, CA) and FITC-conjugated F(ab')₂ goat anti-mouse Ig reagent using a FAC-STAR cell sorter (Becton Dickinson) as described previously.

Preparative regimen and supportive care. All patients, received standardized preparatory regimens. Thirty patients received etoposide (60 mg/kg) IV over 4 hours on day −4 and cyclophosphamide (100 mg/kg) over 2 hours on day −2 with either fractionated total body irradiation (1200 cGy) delivered from day −7 through day −4 or carmustine (15 mg/kg) intravenously over 2 hours on day −6. Five patients with compromised respiratory function received lomustine (9 to 12 mg/kg) PO on day −6 in place of the carmustine. At the time of autologous PBPC and/or bone marrow administration (day 0), each bag was rapidly thawed in a 37°C water bath and infused through a large bore central venous catheter over 5 to 10 minutes. All patients were housed in single rooms with a high efficiency particulate air filtration system. Reverse isolation techniques with masks and gowns were used when neutrophil counts fell below 500/μL. Vancomycin (1 g IV every 12 hours) was initiated on the first day of neutropenia and broad spectrum antibiotics were added for the first febrile episode. All patients received prophylactic low-dose amphotericin (0.15 mg/kg) starting on day +1. All blood products were irradiated with 2,500 cGy before infusion.

Statistical analysis. Randomization was blocked by disease histology (Hodgkin’s and non-Hodgkin’s lymphoma). The primary endpoint was the total number of red blood cell units transfused and the total number of platelet transfusions. The initial trial design called for a total of 60 patients with 30 patients randomized to each treatment arm. The study size was calculated to provide a power to detect a decrease in red blood cell units from 16 to 11 (geometric mean, P < 5%), based on past experience with G-CSF alone. In monitoring the results for safety, an interim analysis at the halfway mark revealed only negligible differences in red blood cell usage, and that favoring the placebo group. A curtailment analysis assuming a 30% advantage for the experimental group was performed. The probability of obtaining a P value less than the 5% in favor of the experimental treatment was found to be very low (1%) and the study was terminated. Statistical analyses for engraftment parameters were performed by t-test, after log transformation for markedly skewed variables (eg, red blood cell units).

RESULTS

Patients. Between February 1992 through July 1993, 35 patients with relapsed Hodgkin’s and non-Hodgkin’s lymphoma undergoing autologous BMT were enrolled. Ten patients declined entry onto this study. Most of the patients were anemic. Patients with non-Hodgkin’s lymphoma had a median hematocrit of 35.7 compared with 32.0 for patients

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with Hodgkin's disease. Patient characteristics are described in Table 1.

Pre-BMT laboratory data. Laboratory data and response to Epo during the 3 weeks before BMT are presented in Table 2. The patients in both groups had normal iron levels at the time of entry onto this study. Both groups also had similar Epo levels and hemoglobin/hematocrit levels. There were no significant changes in hemoglobin and hematocrit from the time of entry onto the study until admission for ABMT (3 weeks later). There was a trend to higher reticulocyte counts in those patients receiving Epo, although this was not statistically different. Patients in both groups were transplanted with similar numbers of mononuclear cells, CFU-GM and burst-forming unit-erythroid.

Engraftment data. Engraftment results are presented in Table 3. No significant differences were noted between the two treatment groups. There were no differences in the total number of red blood cell units transfused nor the number of platelet transfusions were 14 for those receiving placebo and G-CSF (P = .22). Likewise, there were no differences in the time to platelet count ≥20,000/µL without further transfusions, 22 days (15 to 150+) for those receiving Epo and G-CSF compared with 20 days (11 to 54) for those patients receiving placebo and G-CSF (P = .28). No differences could be detected if the two treatment arms were analyzed by disease type, treatment regimen, or by the type of graft. If only the non-Hodgkin's lymphoma patients were compared, the median number of red blood cell transfusions were 14 for those on Epo and 12 for those on placebo.

Toxicity. The treatment protocol was well tolerated. There were no grade 4 toxicities observed that could ascribed to the use of Epo. One patient developed superficial thrombophlebitis involving both the saphenous and the brachial veins 4 days after initiating Epo. Doppler studies did not reveal any deep vein thrombosis. Epo was stopped and the patient did not suffer any further sequelae. No specific systemic organ toxicity occurred in the group receiving Epo compared with the placebo group as measured by the bilirubin and creatinine levels (Table 3). Two patients receiving Epo and one patient receiving placebo developed interstitial pneumonitis after transplantation, and two of these patients died of this complication (one in each group). A third patient (in the Epo group) died of central nervous system bleeding due to refractory thrombocytopenia.

DISCUSSION

Anemia is a universal finding in patients undergoing autologous BMT. The etiology of this anemia is multi-factorial including blood loss, hemolysis, various infections, nutritional factors, bone marrow involvement with disease, and anemia of chronic disease. In addition, myelosuppressive chemotheraphy can cause or exacerbate the anemia associated with the underlying malignancy. Almost all patients who undergo autologous bone marrow transplantation require blood transfusions. Unfortunately transfusions are associated with some risks including that of acute reactions such as fever, chills, and hives and the more chronic problems such as...
as infectious complications and possibly added immunosuppression. Various studies have demonstrated that the use of recombinant Epo results in a statistically significant improvement in the hematocrit with a resultant improvement in the quality of life of selected patients.

Studies of Epo in the setting of BMT have focused primarily on the determination of serum levels in the peri-transplant period. In patients receiving both autologous and autologous marrow transplantation after intensive cytoreduction, an increase in serum levels was observed within 1 to 2 days following the initiation of cytotoxic therapy. The levels are appropriately high during this first week of therapy but soon fall to levels that are not adequate to promote erythroid recovery to normal levels. In another study of autologous transplantation, Epo levels were noted to increase within 7 days of cytoreduction to a mean level of 186 U/L before returning to normal. These results were the rationale for considering the use of Epo before the cytoreductive therapy as well as after BMT. Moreover, we examined whether the addition of Epo to G-CSF would result in a lower number of packed red blood cell units transfused and perhaps a lower requirement for platelet transfusions. We had hoped that the use of Epo in addition to G-CSF would result in prompt erythroid and myeloid engraftment with the potential of improving megakaryocytopenia. Moreover, there are data to suggest that G-CSF enhances the development of early precursors into Epo responsive progenitor cells. In addition, we were also interested in the toxicity and the safety associated with the combination of two recombinant growth factors.

Prior studies using doses of Epo of 3000 to 9000 U to as high as 600 U/kg twice a week to prepare for pre-operative harvesting of autologous red cells have indicated that at least 2 to 3 weeks are required to observe a hematocrit response. Moreover, after the surgery-induced anemia, there was a delay of 7 days before the serum Epo concentration reached its peak in the Epo-treated group compared with controls, suggesting a possible inhibitory effect on endogenous Epo secretion. We had hoped that we might be able to obtain a similar priming effect by giving higher doses of Epo for 3 weeks. In retrospect, it appears that this lead was insufficient to obtain a pre-BMT response to this cytokine. It is possible that patients with the anemia of cancer have higher levels of negative hematopoietic regulators impairing their ability to respond to Epo. Unfortunately, allowing a longer time for pretransplantation Epo therapy was not feasible. Even a 3-week priming period limited the rate of patient accrual; 48 eligible patients were transplanted during the period of this study but could not be enrolled to the trial because the severity of their underlying cancers would not allow waiting to begin high-dose therapy.

It is also possible that administering Epo intravenously did not allow for the most optimal effect. Unfortunately the large doses of Epo that were used in this study precluded the use of the subcutaneous route. Another confounding variable is that Epo may have induced a rapid state of iron deficiency even in those patients whose serum iron levels were normal at the time of entry into the study. Our initial rationale was that blood transfusions would supply the necessary iron for Epo to be effective. Moreover, frequent blood drawing for diagnostic purposes may have added to the difficulty in observing a response. A small pilot study in our unit demonstrated that the diagnostic blood tests and the discards from the central catheter before the blood draws resulted in the loss of approximately 1 U of blood per week (K. Tierney, unpublished observations).

The results suggest that there was no statistically significant difference in the median number of packed red cell blood transfusions administered in either group, as well as the median number of single donor platelet transfusions. We had initially planned to enroll a total of 30 patients in each arm. When we analyzed our data at the mid-way point, however, it became clear that there would not be a statistically significant difference in either arm and there was a trend toward a higher number of platelet transfusions in the group receiving the combination of growth factors.

Although reticulocytosis was somewhat higher late in the post-BMT course, our study did not suggest that the use of Epo for 30 days following transplantation decreased the requirements for red blood cell transfusions during this time. Most of the patients were anemic at the time of entry onto this study since all were receiving chemotherapy for cytoreduction of their underlying disease. These results differ with observations made in prior, nonrandomized studies of Epo following BMT. However, another study combined GM-CSF and Epo in a similar clinical study. The results of this randomized study demonstrated no apparent effect on red blood cell transfusion requirements or platelet recovery. It may not be surprising given that endogenous Epo levels are high after intensive chemotherapy, that the numbers of Epo-responsive progenitor cells are reduced during much of this time, and that even under physiologic conditions, significant Epo-induced increases in hematocrit require several weeks to occur.

Inclusion of patients with Hodgkin's disease may have diluted the net effect of Epo since all of these patients were heavily pretreated with myelotoxic chemotherapy. This lower or lack of response to cytokines is seen with heavily pretreated patients and is not unique to Epo. Alternatively, patients with non-Hodgkin's lymphoma all received monoclonal antibody purging bone marrow. Although monoclonal antibody purging does not seem to affect the CFU-GM and burst-forming unit-erythroid precursor assays, its use is associated clinically with a delay in the time to engraftment of granulocytes. This effect may be enhanced with the use of fractionated total body irradiation as part of the preparatory regimen.

It remains unclear whether there is a role for the use of a combination of Epo and G-CSF in patients undergoing autologous BMT. One possibility to evaluate this question would be to begin Epo earlier, preferably 6 weeks before the ablative regimen. Unfortunately as we have observed, patients undergoing autologous BMT rarely have the possibility for waiting for such a prolonged period of time. A second group of patients who potentially may benefit from this approach are patients who have received less prior chemotherapy and perhaps are more responsive to Epo. In this group of patients, treatment with Epo for 6 to 8 weeks after BMT may be beneficial. A surprising trend was observed in patients with either disease who receive a combination of
Epo and G-CSF in that these patients had a higher platelet transfusional requirement (P = .14). A similar observation has also been made with the combination of Epo and GM-CSF. This possibility raises the concern regarding the use of combination growth factors, especially with the use of hematopoietic growth factors, which have an effect earlier in the differentiation pathway of hematopoietic cells.

In conclusion, the combination of G-CSF and Epo as administered in this study appears to be safe but does not result in an improvement in the total number of red blood cell transfusions or total number of single donor platelet units transfused.

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A randomized study of erythropoietin and granulocyte colony-stimulating factor (G-CSF) versus placebo and G-CSF for patients with Hodgkin’s and non-Hodgkin’s lymphoma undergoing autologous bone marrow transplantation

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