Peripheral Blood Stem Cell Transfusion for Marrow Replacement

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ABSTRACT

A patient is presented who was treated with ablative therapy for Hodgkin’s disease and rescued by reinfusion of peripheral blood stem cells (PBSC). The PBSC were used because previous therapy (chemotherapy and radiation to the pelvis) had resulted in fatty hypocellular marrow which was inadequate for marrow transplantation. The PBSC were collected by leukapheresis before and after recovery of the marrow from suppression with cyclophosphamide to bring the stem cells into cohort cycle and to increase the proportion of stem cells in the peripheral blood for collection. The patient showed a successful recovery on a time scale somewhat longer than previously reported cases, possibly because of the dose of progenitor cells administered, the absence of stimulation by granulocyte macrophage-colony stimulating factor or other cytokine, or potential damage done to stromal elements during previous radiation and chemotherapy. The patient remains in clinical complete remission, fully engrafted, more than one year since his autologous transplant.

Introduction

Ablative therapy with high dose radiation and/or high dose chemotherapy, combined with the administration of autologous bone marrow for rescue from myelotoxic effects, is being used increasingly in an attempt to cure patients with lymphoma and other tumors.1,11,18 Some patients with Hodgkin’s and non-Hodgkin’s lymphoma present with myelophthisis or have hypocellular bone marrows as the result of prior irradiation or chemotherapy. In such patients, it may be helpful to access peripheral blood stem cells (PBSC) that are harvested by leukapheresis to rescue the bone marrow from myeloablative therapy.5,6,8,17

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A case is presented where PBSC rescue of a patient with prior radiation therapy for Hodgkin's disease was performed successfully. Issues include adequacy of stem cell harvesting, time to recovery, and prediction of stem cell yield from the peripheral blood.

Case Report

A 49-year-old Brazilian farmer was diagnosed in April, 1988, as having mixed cellularity Hodgkin's disease that presented as an abdominal mass. He was treated in Sao Paulo, Brazil, with chemotherapy and radiation to the abdomen and pelvis. Owing to the return of discomfort in the back and persistence of the abdominal mass on CT scan, he was referred to the University of Connecticut Health Center/John Dempsey Hospital for consideration of ablative therapy and autologous bone marrow transplantation.

Evaluation in December, 1990, revealed the patient to be a healthy male without outward signs of disease. A bone marrow aspirate and biopsy revealed very hypocellular, fatty marrow, presumably the consequence of prior radiation to the pelvic area.

After discussion of the risks and benefits, approval from the institutional review board, and informed consent, the patient agreed to attempt peripheral blood stem cell collection for rescue after ablative therapy. Three PBSC transfusions were performed without prior stimulation, followed by cyclophosphamide bone marrow suppression and rebound, at which time four PBSC transfusions were performed. The product was frozen in 10 percent dimethylsulfuric acid (DMSO) and stored in vapor phase of liquid nitrogen.

From 2/5/91 to 2/10/91, the patient received ablative chemotherapy (etoposide 200 mg per M\(^2\) BID for four days, carboplatin 400 mg per M\(^2\) QD for four days, cyclophosphamide 900 mg per M\(^2\) BID for four days, MESNA 12 mg per kg for 13 doses). On 2/12/91 the patient received a thawed pool of cells from all of the prior PBSC transfusions (total estimated dose was 5.4 \(\times 10^{10}\) MNC).

The patient tolerated his chemotherapy well with only minimal amounts of nausea and vomiting, although he required a short course of hyperalimentation. Vancomycin 1 gram IV every 12 hours was administered for 14 days, and cefazidime 2 grams IV every eight hours for 13 days was given for fever. All cultures were negative. On 3/6/91, the patient was sufficiently recovered to be discharged and followed in the Ambulatory Care Clinic, as his hematologic parameters gradually returned to normal levels (figure 1). He returned home to Brazil on 4/11/91 and continues to do well.

Methods

COLLECTION OF PBSC

From 1/2/91 to 1/4/91 three PBSC transfusions* were performed, each extracting mononuclear cells from the equivalent of the patient's circulating blood volume. The collected stem cells were frozen in 10 percent DMSO and vapor phase liquid nitrogen. On 1/5/91, the patient received cyclophosphamide (3 g per M\(^2\))

* Fenwal CS 3000, Baxter Healthcare Corp., Fenwal Division, Deerfield, IL 60015.
to synchronize stem cell production. Over the next three weeks, the blood counts increased to levels sufficient to continue PBSC collections. On 1/28/91, blood was obtained for complete blood count, CD34 marker studies and stem cell culture, and on 1/29/91 a series of four PBSC transfusions was begun.

FLOW CYTOMETRY ANALYSIS OF CD34+ CELLS

Flow cytometric analysis† of PBSC level on 1/28/91 was performed using murine monoclonal anti CD34 (HPCA per My10)‡ and goat anti-murine Ig tagged with fluorescein isothiocyanate (FITC).† Mononuclear cells obtained by density gradient centrifugation were suspended in cold RPMI culture medium. For staining, 20 μl of murine anti-CD34 was incubated with 10⁶ cells for 45 minutes at 2 to 8°C. The cells were washed twice in cold PBS and 10⁶ cells were incubated for 30 minutes with one μl of goat anti-murine Ig tagged with FITC F(ab')₂. Flow cytometric analysis was performed with bit maps around the lymphoblast fraction. Nonspecific uptake of primary antibody was determined by using mouse Ig from unstimulated mice.

Results

ASSESSMENT OF THE FREQUENCY OF CD 34 POSITIVE CELLS IN PERIPHERAL BLOOD

A whole blood sample drawn on 1/28/91 contained one percent of the MNC marked with CD 34 (1.8 × 10⁴ PBSC per ml blood). Presuming this ratio remained unchanged through freezing, storage, and thaw phases, on 2/12/91 the patient received by infusion a total of 5.4 x 10⁸ CD 34 + cells: 5.4 × 10⁶ per kg corrected for patient weight of 100 kg.

ASSESSMENT OF CLINICAL RECOVERY

The patient began feeling better with return of appetite on or about 2/28/91. By 3/6/92 the patient was feeling well enough to be discharged and was showing signs of early hematologic recovery.

RECOVERY OF HEMATOPOIESIS

Recovery of the patient's marrow occurred shortly after discharge with the neutrophil level increasing from 70 per μl on 3/5/91 to 525 per μl on 3/18/91 and 1365 per μl on 3/28/91 (table I). Platelets responded similarly resulting in a timetable with a steady increase, unsupported by platelet transfusion, beginning at 33,000 on 3/14/91, increasing to 50,000 on 3/21/91 and almost 100,000 on 4/4/91. Overall hematologic

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† Coulter Epics C, Coulter Electronics, Hialeah, FL 33010.
‡ Becton Dickinson Immunocytometry Systems, Mountain View, CA 94039.
recovery as indicated by the last red cell transfusion was 41 days after stem cell transplantation and the reticulocyte count finally increasing to greater than 1.0 percent on 3/18/91. On 3/11/91 the total leukocyte count had increased to greater than 1,000, but virtually all the cells seen were classified as lymphocytes.

Discussion

It is well known that hematopoietic stem cells circulate in the peripheral blood. Accordingly, peripheral blood is a potential source of stem cells for transplantation. Complete hematopoietic reconstitution following autologous transplantation with PBSCs were first described in 1986. Since that time, transplantation of PBSC has been utilized increasingly to rescue patients from myeloablative chemoradiotherapy administered for hematologic malignancies and solid tumors.

Myelosuppressive chemotherapy followed by apheresis for collection of circulating progenitor/stem cells during the phase of hematopoietic recovery has been utilized to improve the yield of stem cells, but optimal timing of collection following these stem cell mobilization procedures is difficult to predict. In the present study, the number of white blood cells, mononuclear cells, and platelets were utilized to determine the optimal time for PBSC and quantification of stem cells was attempted by measuring the number of CD34-positive cells. From the CD34 measurement and the formula developed by Siena et al, the patient’s blood contained approximately 1,540 CFU-GM per ml at the time of collection. In contrast to the usual post-transplant hematopoietic recovery, our patient exhibited a prolonged postablative hematopoietic recovery period. This may have been due, in part, to heavy pretreatment of our patient with chemotherapy to maintain disease control. Hematopoietic stem cells or stromal support cells may be damaged, thereby impairing the proliferative and differentiation responses. Accordingly, GM-CSF therapy prior to peripheral blood stem cell collection might have enhanced the yield. Cells collected by Siena et al were induced with GM-CSF in vivo and following infusion, which may have shortened the time interval to differentiation. It is predictable that hematopoietic recovery utilizing growth factor primed progenitor/stem cells will be accelerated. Alternatively, it is possible that intense chemoradiotherapy in our patient damaged stromal cells that are known to release paracrine growth factors into the microenvironment that may in turn, enhance differentiation and growth.

Summary

In summary, a case is presented where PBSC transplantation in a patient with prior radiation therapy was effective in rescuing the patient from intense chemotherapy. Measurement of CD34-positive cell frequency in the peripheral blood may be helpful as a rapid method to determine the optimal time for apheresis. The clinical course in this patient underscores the potential importance of stem cell dose and the condition of the receiving marrow stromal elements in the hematopoietic recovery following PBSC transplantation.

Acknowledgment

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References


