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The Possible Cost Effectiveness of Peripheral Blood Stem Cell Mobilization with Cyclophosphamide and the Late Addition of G-CSF

The purpose of this study was to develop a cost-effective protocol for the mobilization of peripheral blood stem cells (PBSC) in patients with malignancy. Thirty consecutive patients were randomized to mobilize PBSC with the late addition of a standard 250 μg dose of G-CSF (Neutrogen®) from day 8 or early addition of the same dose of G-CSF from day 2, following cyclophosphamide (CY) 4 g/m^2. The median yield of CD34+ cells from evaluated patients was 7.87×10^6/kg (range, 2.06-27.25), collected in a median of four apheresis (range, 2-9). Target CD34+ cell doses $\geq2.0\times10^6$/kg were achieved in all patients able to be evaluated. There were no statistically significant differences in CD34+ cell yields or toxicities. Overall engraftment occurred with median days to neutrophils $>0.5\times10^9$/L or platelets $>20\times10^9$/L of 11 and 17 days, respectively. However, the duration of G-CSF administration was markedly shorter in the late use of G-CSF group than in the early use of G-CSF group, with a median of 9 days compared with 15 days ($p<0.001$). PBSC harvesting after priming with CY plus delayed use of G-CSF made it a safe and cost-effective procedure.

Key Words: *Stem Cells; CD34 Antigen; Hematopoietic Stem Cell Transplantation; Granulocyte Colony-Stimulating Factor*

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INTRODUCTION

High-dose chemotherapy facilitated by autologous stem cell support is being more frequently applied in the treatment of cancer. A major concern in this treatment strategy is how to obtain sufficient peripheral blood stem cells (PBSC) to ensure hematopoietic engraftment. There is general agreement that the highest stem cell yields are achieved with a combination of chemotherapy and growth factor (1).

However, protocols used to mobilized PBSC vary from center to center. Especially, there is no agreement as to the optimal dosing schedule of G-CSF to mobilize PBSC. Doses ranging from 2 μg/kg/day to 60 μg/kg/day, starting at different time points prior to PBSC collection have been associated with PBSC mobilization (2, 3).

The use of G-CSF is a major expense in the mobilization of PBSC. Accordingly, we have investigated a safe and cost-effective protocol for the mobilization of PBSC in patients with malignancy. Therefore, we designed a randomized clinical trial comparing 'late' administration of G-CSF (day 8) vs 'early' administration of G-CSF (day 2) following 4 g/m^2 cyclophosphamide (CY).

MATERIALS AND METHODS

Patients

Between June 1997 and January 1999, 30 patients with breast cancer, non-Hodgkin's lymphoma, multiple myeloma and Ewing's sarcoma were enrolled in the study. Characteristics of the 30 patients are shown in Table 1.

Table 1. Patients characteristics

	Group A (Day 8)	Group B (Day 2)
No. of patients	15	15
Age: years (median, range)	40 (20-65)	39 (22-56)
Sex (male/female)	6/9	6/9
Disease (Patient No.)		
Breast cancer	8	8
Non-Hodgkin's lymphoma	4	5
Multiple myeloma	2	1
Ewing's sarcoma	1	1
Cycles of chemotherapy before mobilization median, range	7 (2-21)	5 (1-16)
No. of patients with prior radiotherapy	5	3

Patients were eligible for inclusion, if they were <65 years of age, had adequate renal function and were potential candidates for high dose chemotherapy and PBSC transplantation for poor prognosis malignancy.

The patients were randomized into two groups. Group A (n=15) started G-CSF on day 8 after CY infusion while patients in group B (n=15) began G-CSF on day 2 after CY infusion. There were no significant differences between patient characteristics in these two groups. Collected CD34+ cell counts were not available for one patient in group A because of a malfunction in the flow cytometry. Thus, data are available for only 29 patients.

PBSC mobilization and collection

All patients received a combination of CY followed by G-CSF (Neutrogen; Choongwae Ltd, Seoul, Korea) at a standard dose of 250 μg/day subcutaneously, commencing 2 days or 8 days after CY, and continuing until a day prior to the final PBSC harvest. CY was administered at a dose of 4 g/m^2 intravenously (IV) over 2 hr with subsequent IV mesna 4 g/m^2 in five divided doses as urothelial prophylaxis.

The first PBSC harvest was performed on the day after the WBC had exceeded 3×10^9/L. PBSC were collected using a Baxter CS3000 plus cell separator. Each apheresis procedure was performed for approximately 2-4 hr. Seven to 14 L were processed with the Baxter CS3000 plus. The total mononuclear cell count, CD34+ content of the leukapheresis product, was monitored daily following each collection.

Immunofluorescence staining and flow cytometry

For immunofluorescence analysis, 100 μL of the mobilized peripheral blood was incubated for 30 min at 4℃ with phycoerythrin-conjugated monoclonal antibody CD34 (HPCA-2, Becton Dickinson). Isotype-specific antibodies served as control. Immunofluorescence analysis was performed using a two-parameter FACScan (Beckton Dickinson).

Statistical analysis

The data are given as the median and range. Testing the significance of differences in ordinal data between the two groups were done with the non-parametric Wilcoxon rank sum test, and differences between proportions were calculated with the Fisher's exact test.

$p<0.05$ was considered significant.

Statistical analysis was performed using the Statistical Package for Social Scientists (SPSS Inc, Chicago, IL, U.S.A.).

RESULTS

PBSC mobilization and collection

The median yield of CD34+ cells for all patients evaluated was 7.87×10^6/kg (range, 2.06-27.25), collected in a median of four apheresis (range, 2-9). Target CD34+ cell doses more than 2.0×10^6 CD34+ cells per kg of body weight were obtained from the evaluated patients. Table 2 summarizes the comparisons of the two groups. The median number of CD34+ cells harvested was not significantly different between the two groups (p=0.974). The median duration from the administration of CY to the initiation of apheresis was longer in group A than in group B (14 vs 12 days) (p=0.025). However, the median duration of G-CSF use for PBSC harvest was significant shorter in group A than in group B (9 vs 15 days) (p<0.001).

Toxicities

The mobilization protocol tolerated well. Hematologic and clinical toxicities are shown in Table 3. There were no significant differences in the duration or depth of neutropenia and thrombocytopenia in the two groups.

Seventeen of 29 (59%) experienced febrile neutropenia (one patient had positive blood cultures); 6 of 14 (43%)

Table 2. Leukapheresis collection data

	Group A (Day 8)	Group B (Day 2)
Procedure per patient	3.5 (2-5)	4 (2-9)
MNC ($\times10^8$/kg)	5.08 (2.3-6.65)	4.78 (2.14-8.35)
CD34+ cells ($\times10^6$/kg)	6.76 (2.06-24.8)	8.5 (2.47-27.25)
Days between D1 and collection*	14 (10-19)	12 (10-17)
Days of G-CSF application†	9 (6-16)	15 (10-19)

Data are expressed as median value (range)
*p=0.025, †p<0.001 (Wilcoxon rank sum test)
MNC, mononuclear cells

Table 3. Hematological & clinical toxicities

	Group A (Day 8)	Group B (Day 2)
ANC nadir ($\times10^9$/L)	0.02 (0-0.6)	0.01 (1-0.14)
Days of ANC <0.5$\times10^9$/L	4 (0-8)	4 (3-8)
% of Pt. with fever	43	73
Platelet nadir ($\times10^9$/L)	33 (12-124)	45 (15-93)
Days of platelets <50$\times10^9$/L	3 (0-22)	3 (0-8)
% of Pt. requiring platelet transfusion	50	47
% of Pt. requiring RBC transfusion	36	33

Data are expressed as median value (range)
ANC, absolute neutrophil count; Pt, patients; RBC, red blood cell

Table 4. Engraftment data for 23 patients receiving high-dose chemotherapy and peripheral blood stem cell transplantation

	Group A (Day 8)	Group B (Day 2)
Days of ANC $>0.5\times10^9$/L	11 (9-31)	11 (9-22)
Days of ANC $>1.0\times10^9$/L	12 (10-32)	12 (10-22)
Days of Platelet $>20\times10^9$/L	19 (10-54)	16 (10-46)
Days of In-patient stay	28 (14-53)	29 (14-50)
Transfusion		
Units of red cell	4 (0-8)	4 (2-12)
Units of platelets	12 (3-96)	21 (2-80)
Days antibiotics	12 (0-21)	10 (6-30)

Data are expressed as median value (range)
ANC, absolute neutrophil count

in group A and 11 of 15 (73%) in group B (p=0.1). Ten of 29 (35%) received red cell transfusion and 14 of 29 (48%) received platelet transfusion, but there were no significant differences between the two groups. Mucositis and other extramedullary toxicity were generally mild.

Engraftment

All but six patients proceeded to high-dose chemotherapy with autologous stem cell transplantation. In six patients an elective clinical decision not to proceed was made. For the 23 patients transplanted, the median dose of CD34+ cells reinfused was 8.14×10^6/kg (range, 2.06-24.8). There were no procedure-related deaths after high-dose chemotherapy. Engraftment data for all 23 patients is shown in Table 4. There were no significant differences in the engraftment results between two groups.

DISCUSSION

This study demonstrates a safe and cost-effective protocol for the mobilization of PBSC in patients with malignancy. A number of methods have been used to mobilize stem cells but currently it is not clear that any regimen is more optimal than another. Although chemotherapeutic agents that, are effective for underlying tumor, have been used successfully, non-myeloablative and non-stem cell toxin drugs such as cyclophosphamide (CY) is typically used as the chemotherapy agent for PBSC mobilization (4).

As PBSC increase appears to be related to the intensity of the preceding myelosuppression, higher doses of CY are associated with higher stem cell yield. However, increasing the dose of CY is also associated with significant morbidity and occasional mortality (5, 6). Meanwhile, it is clear that the additional amplification, observed when G-CSF or GM-CSF is given following chemotherapy, allows more stem cells to be collected with fewer leukaphereses (7, 8). Some investigators have suggested that increasing the dose of G-CSF increases the yield of CD34+ cell (3, 9). In this study, there were also more CD34+ cells collected in the early use of G-CSF group than in the late use of G-CSF group but these differences were small and not statistically significant.

Higher progenitor cell doses were also associated with more predictable hematopoietic reconstitution (10). However, there is no linear relationship between CD34 numbers obtained and engraftment. This is most likely due to a "threshold effect" (11, 12). In general, a threshold of $\geq2.0\times10^6$ CD34+ cells is recommended to ensure hematopoietic recovery (13). In our study, more than 2.0×10^6 CD34+ cells/kg were harvested from patients who were involved in the late use of G-CSF group with a median of four apheresis procedures and the median number of CD34+ cells harvested was 6.76×10^6/kg in those patients.

Meanwhile, using a higher dose of G-CSF increases the expense. So, the use of G-CSF adds greatly to the cost of the current PBSC mobilization technique (14). In our study, the duration of G-CSF administration was markedly shorter in the late use of G-CSF group than in the early use of G-CSF group, with a median of 9 days compared with 15 days. The delayed addition of a standard dose of G-CSF presents the potential for saving on the cost of mobilization when compared to the addition of G-CSF at day 2. Other published studies have reported results similar to our data (14).

It is also important to note that the early use or increasing dose of G-CSF paradoxically causes the development of severe neutropenia, illustrating the maturation effect (early cell death) of G-CSF on the mature cells which survive the CY therapy (15). Therefore, it is suggested that the delayed and reduced G-CSF can prevent severe neutropenia (2, 12). However, our results have shown that there were no significant differences in neutrophil nadir between patients receiving G-CSF at day 8 or day 2 after CY infusion.

Although, due to limited number of patients used in our study, our results is not conclusive, it is clear that CY 4 g/m^2 followed by G-CSF (Neutrogen®) 250 μg/day starting at day 8 can obtain adequate CD34+ cell yields with tolerable toxicities.

Finally, this study has shown that PBSC harvesting after priming with CY plus delayed use of G-CSF can be a safe and cost-effective procedure.

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