Abstract  
Objective: The aim of this study was to clarify the appropriate timing for peripheral blood progenitor cells (PBPC) harvesting after platinum-based mobilization chemotherapy by measurement of the circulating CD34+ cell concentrations.  
Patients and Methods: PBPC were collected for autotransplantation via a total of 68 leukaphereses in 16 patients with gynecological cancer. Circulating CD34+ cell concentrations were measured by CD34-side scatter parameter analysis.  
Results: Data could be fitted into a linear regression line described by the equation y=0.33+4.14\times x (R^2=0.256), where y=the number of harvested colony-forming unit granulocyte macrophage (CFU-GM (\times 10^5/kg) and x=the percentage of circulating CD34+ cells and y=1.747+44.53\times x (R^2=0.475), where y=the number of harvested CD34+ cells (\times 10^6/kg) and x=the percentage of circulating CD34+ cells. Failure to mobilize sufficient CFU-GM numbers (>1\times 10^5/kg) occurred in 29 of 31 leukaphereses when the percentage of circulating CD34+ cells was less than 0.10%.  
Conclusions: Harvesting procedure may be avoided when circulating CD34+ cells showed less than 0.10% after platinum-based mobilization chemotherapy.  

Keywords PBPC harvest · CD34 · CFU-GM · Platinum-based chemotherapy

Introduction

Over the past several years, high-dose chemotherapy regimens have been widely used in the treatment of solid and hematologic tumors. Large numbers of peripheral blood progenitor cells (PBPC) can be mobilized and collected following administration of growth factors, chemotherapy, or both, and reinfused with or without autologous marrow following high-dose chemotherapy.  
Large quantities of PBPC can be harvested by a practical number of leukaphereses in a reasonable time, i.e., two to five daily leukaphereses for 2–5 consecutive days, only at the time of marrow recovery from cancer chemotherapy-induced pancytopenia. However, few reports are available demonstrating that platinum-based chemotherapy, which produces satisfactory antitumor activity for gynecological malignancies, mobilized a sufficient number of PBPC [11, 14, 15]. In platinum-based mobilization chemotherapy, the optimal time to perform the PBPC collections may vary from patient to patient, which makes scheduling of leukapheresis logistically difficult. Furthermore, some patients may not have mobilization of hematopoietic progenitors into the circulation, which leads to a large number of leukaphereses.  
The aim of this study was to clarify the appropriate timing for PBPC harvesting after platinum-based mobilization chemotherapy by measurement of the circulating CD34+ cell concentrations. Our results will help to determine the appropriate timing for PBPC harvesting and reduce the number of leukapheresis procedures needed to achieve sufficient numbers of PBPC in platinum-based mobilization chemotherapy.

Patients and methods

Patients characteristics

Sixteen patients (Table 1) were enrolled into this study. There were 13 patients with ovarian cancer, one with tubal cancer, one with choriocarcinoma, and one with a placental site trophoblastic tumor. Their ages were between 21 and 61 years. Patients were required to have a creatinine clearance rate greater than 60 ml/min and GOT, GPT, and bilirubin less than twice the normal levels. Minimum hematologic requirements included a WBC count greater than 3\times 10^9/L and a platelet count greater than 100\times 10^9/L. All patients provided written informed consent and had an Eastern Cooperative Oncology Group performance status of 0–1 at the time of study entry.
To enhance PBPC collection, recombinant human granulocyte colony-stimulating factor (rhG-CSF: Kirin Brewery Co., Ltd., Tokyo, Japan) was given as a daily intracutaneous infusion of 50 µg/m2 starting at the nadir of WBC after chemotherapy.

A colony-forming assay was performed using two different media. 50,000 mononuclear cells or 1 µl of collected PBPC were suspended in 1 ml of complete MEM-alpha medium [9, 10] or MethoCult GF H4434V medium (Veritas Co., Tokyo, Japan) and cultured in a Petri dish (Corning 25000). After 14 d of incubation at 37°C, with 5% CO2 and high humidity, the CFU-GM consisting of 40 or more cells was counted under an inverted microscope and averaged. The two techniques yielded results of the same order of magnitude (data not shown).

PBPC were collected for autotransplantation via a total of 68 continuous-flow leukaphereses in 16 patients with gynecological cancer after platinum-based mobilization chemotherapy with rhG-CSF. The median number of CFU-GM and CD34+ cells collected in a single leukapheresis were 0.73x10^6/kg (range 0–3.14) and 6.57x10^4 cells/kg (range 0.94–27.3), respectively. No difference was observed regarding the CFU-GM and CD34+ cells yield between various chemotherapy regimens (data not shown). Ten patients received high-dose chemotherapy with PBPC transplantation. The median infused CFU-GM dose was 2.5x10^5/kg (range, 0.75–5.2). Hematopoietic engraftment was done in all patients at a median of 10 days (range, 8–12 d) to achieve neutrophils greater than 0.5x10^9/L.

Figure 1 shows representative immunofluorescence analysis of CD34 antigen expression using the side scatter analysis method. CD34+ cells were easily detected in spite of the small number of positive events (0.07%: 666/1x10^6 cells). Fig. 2 shows the relationship between the percentage of peripheral blood CD34+ cells and harvested CFU-GM/kg. Data could be fitted into a linear re-