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Large-scale Collection of Circulating Haematopoietic Progenitors in Cancer Patients Treated with High-dose Cyclophosphamide and Recombinant Human GM-CSF

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Circulating haematopoietic progenitors from 36 cancer patients were collected by continuous-flow leukapheresis during the phase of rapid haematopoietic recovery after pancytopenia induced by high-dose cyclophosphamide and then cryopreserved for autologous transplantation. 20 of the patients also received intravenous infusion of recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) for 7, 10 or 14 days after cyclophosphamide. 106 leukapheresis procedures were done for 2–5 consecutive days. Leukapheresis was started significantly earlier in patients receiving rhGM-CSF. In these patients, yields of peripheral blood elements (leucocytes, mononuclear cells, haematopoietic progenitors and platelets) were significantly higher than in controls treated with cyclophosphamide only. In particular, the mean number of granulocyte–monocyte colony-forming cells was 43.88 × 10^4 vs. 6.16 × 10^4 per kg patient body weight per leukapheresis. Side-effects of leukapheresis were limited to central venous catheter occlusion and fever in 4% and 2% of all procedures, respectively. *Eur J Cancer*, Vol. 26, No. 5, pp. 562–56, 1990.

INTRODUCTION

HAEMATOPOIETIC progenitor cells can be retrieved from the circulation, cryopreserved and used to reconstitute the haematopoietic system of cancer patients after myeloablative chemoradiotherapy [1]. Advantages of autografting with circulating rather than with bone-marrow haematopoietic progenitors include the possibility of avoiding general anaesthesia, the lower risk of contaminating tumour cells [2] and possibly the faster haematopoietic reconstitution of myeloablated hosts [1]. However, haematopoietic reconstitution has been incomplete in some patients autografted with circulating cells, possibly because of an insufficient dose of progenitors. [3]. Several procedures, including chemotherapy [4] and treatment with recombinant human granulocyte (G-CSF) [5] or granulocyte–macrophage colony stimulating factor (rhGM-CSF) [6] increase the number of circulating haematopoietic progenitors. In particular, high-dose cyclophosphamide can increase by about 30-fold the number of circulating progenitors during the phase of rapid recovery after drug-induced pancytopenia [7–9]. Circulating haematopoietic progenitors collected after cyclophosphamide can be used to accelerate autologous haematopoietic reconstitution after myeloablative chemoradiotherapy [7, 8]. In addition, rhGM-CSF increases the number of haematopoietic progenitors in the peripheral blood of patients treated with high-dose cyclophosphamide by about 160-fold compared with steady-state values [9, 10]. We describe the methodology, side-effects and cell yields of leukapheresis for large-scale collection of haematopoietic progenitors from patients treated with high-dose cyclophosphamide or without rhGM-CSF.

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PATIENTS AND METHODS

Patients and therapy

The characteristics of the 36 patients evaluated in this study are shown in Table 1. 5 patients with non-Hodgkin's lymphoma had received at least two-drug chemotherapy. In all cases bone marrow involvement was ruled out by morphological and immunological analysis of iliac crest marrow aspirate and/or biopsy specimen. After patients had given written informed consent, they were treated according to high-dose sequential chemotherapy protocols approved by the institute's committee for clinical investigation.

Table 1. Patients' characteristics

	Treatment group	
	Cyclophosphamide*	Cyclophosphamide plus rhGM-CSF
No. of patients	16	20
Median age (range)	40 (25–53)	40 (21–53)
M/F	3/13	2/18
Diagnosis		
Non-Hodgkin's lymphoma	6	8
Breast cancer†	8	12
Small cell lung cancer	2	0
Previous chemotherapy	3	2

*High-dose.
†11 patients with operable breast carcinoma with ten or more metastatic axillary nodes and 9 patients with inflammatory breast carcinoma.

On day 0 of therapy, all patients were treated with 7 g/m^2 cyclophosphamide divided in five doses given intravenously over 1 h every 3 h. For urothelial protection, patients were given an intravenous bolus of 2-mercaptoethane sulphonate every 3 h (1.5 g for five doses and 1.0 g for seven additional doses). Starting 24 h after cyclophosphamide, 20 patients received rhGM-CSF (Sandoz/Schering Plough) 5.5 μg of protein per kg daily by continuous intravenous infusion either for 7 days from day +1 to +7 (3 patients), for 10 days from day +1 to +10 (3), for 10 days from day +5 to +14 (3) or for 14 days from day +1 to +14 (11) after cyclophosphamide. 16 control patients did not receive rhGM-CSF. Antitumour effects of high-dose cyclophosphamide are reported elsewhere [11].

Continuous-flow leukapheresis

During the haematopoietic recovery after cyclophosphamide-induced pancytopenia, peripheral blood and bone-marrow nucleated cells were collected and cryopreserved as a source of haematopoietic progenitors to support subsequent myeloablative treatments. Starting on the first day that the leucocyte count reached 1000/μl and platelets 70,000/μl, patients underwent peripheral blood leukaphereses on 2–5 consecutive days.

A continuous-flow blood-cell separator (IBM-COBE 2997, Lakewood, Colorado) at a flow-rate of 30–45 ml/min was used. For anticoagulation ACD-Formula A (Baxter, Trieste) solution was added to the blood entering the circuit at 1:8 to 1:10. To prevent hypocalcaemia-related symptoms, 10–40 ml 10% calcium gluconate was given intravenously throughout leukapheresis. The centrifuge speed was adjusted to 700–950 revolutions per min to obtain a visible buffy-coat layer. Leukapheresis lasted for 3.5 h (first 30 min for formation of buffy-coat cell layer). Optimal collection of mononuclear cells was achieved by adjusting the buffy-coat aspiration rate to 0.5 ml/min for the first hour, to 1.0 ml/min for the second hour and to 1.5 ml/min for the third hour of leukapheresis. The total blood volume processed per leukapheresis was between 7.2 and 9.9 l. Buffy-coat cells were collected into transfer bags (Baxter) containing 50 ml Hank's balanced salt solution (Gibco) and 30 IU/ml heparin. This process resulted in 230–250 ml harvested cell suspensions which were cryopreserved for subsequent transplantation in 90% heat-inactivated human serum/10% dimethylsulfoxide without further cell fractionation [7].

For blood aspiration we used either an antecubital vein or the 14 G lumen of a double-lumen heparin-coated Vialon catheter (Deltacath, Deseret Medical, Sandy, Utah) in 24 (67%) and 12 (33%) patients, respectively. For blood reinfusion we used the 18 G lumen of a central venous catheter. The flow efficiency of venous catheters was maintained by weekly prophylactic 30 min infusion with 20,000 IU/ml urokinase. Cell counts, including differential, were done by an H6000 cell counter (Technicon).

Evaluation of haematopoietic progenitor cells

Day 14 granulomonocytic-colony forming units (CFU-GM) were counted by plating aliquots of leukapheresis in appropriate culture conditions in semisolid medium [12]. CFU-GM were evaluated in 39 of 45 (87%) leukapheresis procedures from patients not receiving rhGM-CSF and in 55 of 61 (90%) procedures from patients receiving rhGM-CSF after high-dose cyclophosphamide.

RESULTS

Tolerability of leukapheresis

106 leukapheresis procedures were done in the 36 patients (median 3 per patient, range 2–5) on 2–5 consecutive days. Procedures were completed in all cases. Four procedures (4%) were complicated by central venous catheter occlusion, which was reversed by flushing with 20,000 IU/ml urokinase for 15 min. In subsequent cases, prophylactic treatment of central venous catheter with urokinase before leukapheresis guaranteed venous patency. In 2 cases (2%), both receiving rhGM-CSF during leukapheresis, chills and temperatures of 39.5–40°C were observed. These symptoms subsided after oral administration of paracetamol.

Effect of rhGM-CSF on timing of leukapheresis

Starting on day +5 to +7 after cyclophosphamide, patients had drug-induced pancytopenia for 7–11 days depending on whether or not they received rhGM-CSF [11]. The mean day of first leukapheresis was day +16.9 (range 13–20) after cyclophosphamide for patients who did not receive rhGM-CSF. Patients receiving rhGM-CSF underwent first leukapheresis significantly earlier than controls given cyclophosphamide only: mean day +14.3 (11–19) ($P = 0.0075$, two tailed t test).

Effect of rhGM-CSF on yield

45 leukapheresis samples were obtained from 16 patients who did not receive rhGM-CSF after high-dose cyclophosphamide. 61 samples were obtained from 20 patients receiving rhGM-CSF. Mean values per leukapheresis sample of harvested leukocytes, mononuclear cells, CFU-GM and platelets are shown in Table 2. Treatment of patients with rhGM-CSF significantly increased the yield of peripheral blood elements per leukapheresis sample, while erythrocyte content was similar in all treatment groups (2.79 and 2.69% haematocrit in leukapheresis samples from patients treated or untreated with rhGM-CSF, respectively).

The mean number of CFU-GM per kg body weight per leukapheresis significantly differed between rhGM-CSF treated

*Table 2. Yield of peripheral blood elements**

rhGM-CSF	No. of leukapheresis procedures	Leucocytes (× 10^9)	Mononuclear cells (× 10^9)	CFU-GM (× 10^6)	Platelets (× 10^{11})
None (n = 16)	45	5.2 (2.8)	2.7 (1.3)	3.3 (3.1)	2.4 (1.2)
Days 1–7 (n = 3)	10	7.0 (2.6)	2.7 (1.0)	9.6 (4.9)†	5.2 (2.1)†
Days 1–10 (n = 3)	8	9.0 (3.7)	4.2 (1.0)†	10.5 (2.1)†	4.2 (1.6)
Days 5–14 (n = 3)	12	9.7 (4.3)†	4.6 (2.4)	29.1 (27.3)†	4.3 (1.8)
Days 1–14 (n = 11)	31	14.7 (8.5)†	4.8 (1.9)†	26.4 (20.9)†	3.0 (1.3)

*Mean (S.D.) in 230–250 ml leukapheresis cell suspensions. On day 0 of therapy, all patients received high-dose cyclophosphamide.

†$P < 0.001$ in comparison with control patients treated with cyclophosphamide without rhGM-CSF.

and untreated patients (43.88×10^4/kg vs. 6.16×10^4/kg, $P = 0.0001$, two-tailed t test).

Effect of schedule rhGM-CSF

The yield of mononuclear cells and CFU-GM per leukapheresis sample was analysed according to the schedule of rhGM-CSF administration (Table 2). The yield of mononuclear cells was higher in leukapheresis procedures from patients receiving days 1–10, 5–14 and 1–14 rhGM-CSF schedules compared with controls and with the day 1–7 schedule. Overall the number of harvested CFU-GM haematopoietic progenitors was higher in procedures from rhGM-CSF treated patients. In particular, days 5–14 and 1–14 schedules resulted in the highest yield of CFU-GM.

DISCUSSION

We have described a reproducible, efficient and safe method for large-scale collection of circulating haematopoietic progenitors by continuous-flow leukapheresis from cancer patients treated with high-dose cyclophosphamide. We also showed that in the same patients rhGM-CSF at 5.5 μg/kg daily, a dosage associated with occasional mild side-effects [10, 12], significantly increased the yield of harvested progenitors useable for autografting. We administered rhGM-CSF in different schedules to establish the quickest haematopoietic recovery after cyclophosphamide. In particular, patients given rhGM-CSF from days 1–14 recovered leucocyte and platelet counts significantly earlier than patients given other schedules or controls [11]. The yields of haematopoietic progenitors were highest with days 5–14 and 1–14 rhGM-CSF schedules. Thus, rhGM-CSF on days 1–14 is the best schedule.

The administration of rhGM-CSF after cyclophosphamide increased the absolute number of circulating mononuclear cells and CFU-GM progenitors collectable by leukapheresis. Moreover, rhGM-CSF given from days 1–14 and 5–14 after cyclophosphamide increased the ratio between CFU-GM and mononuclear cells in harvested cell suspensions. This observation indicated that administration of rhGM-CSF during leukapheresis enriches the mononuclear cell fraction. In previous studies we found that these circulating progenitors possess heterogeneous differentiation cell-surface characteristics, ranging from the most undifferentiated cells (CD34+, CD33−) to multipotent and unipotent colony-forming cells (CD34+, CD33+) [9].

In consecutive patients, combined transplantation of autologous bone marrow and peripheral blood leucocytes harvested after high-dose cyclophosphamide and rhGM-CSF allowed even faster and more complete recovery of all haematopoietic lineages and better treatment tolerance than has been described previously with the same method but without rhGM-CSF [13]. A possible explanation is that committed as well as early multipotent haematopoietic progenitors are retrieved from the circulation and transplanted in addition to marrow cells after myeloablation [9]. It has been proposed that a threshold of $15–50 \times 10^4$ CFU-GM per kg be transplanted to ensure complete and durable engraftment after myeloablative therapy [1]. For an average 60 kg patient three leukapheresis procedures harvested as described might be sufficient for reconstituting the haematopoietic system after myeloablation.

The circulation of haematopoietic progenitors with or without rhGM-CSF is transient and dynamic, occurring in the second and third week after intensive chemotherapy [7, 9, 13]. However, leukapheresis after intensive chemotherapy has no deleterious effect on haematopoietic recovery, measured by the rates of rise of neutrophils, platelets and lymphocytes during and after the procedure. The leukapheresis technique was well tolerated, being associated with minor and reversible side-effects in less than 5% of procedures. Furthermore venous access never limited a 30–45 ml/min blood flow which in all cases was sufficient to achieve the cell yields we reported.

The wide variations of granulocyte content, 15–70% of harvested leukapheresis suspensions, reflected the granulocytosis observed in the peripheral blood during the rebound phase after cyclophosphamide, especially when treatment with rhGM-CSF is used. The technical characteristics of the continuous-flow blood-cell separator we used did not allow us to collect cell populations that were less contaminated by granulocytes. However, we have not observed clumping during thawing of leukapheresis bags, even at the highest leucocyte counts.

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