Pergamon

European Journal of Cancer Vol. 32A, No. 11, pp. 1938–1942, 1996
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Printed in Great Britain
0959-8049/96 $15.00 + 0.00

PII: S0959-8049(96)00209-2

Original Paper

High-dose Melphalan Followed by Autograft Employing Non-cryopreserved Peripheral Blood Progenitor Cells in Children

N. Jones,[1] D. Williams,[1] V. Broadbent,[1] K. Jestice,[2] P. Boraks,[2] M. Scott,[2] S. Ager[2] and R. Marcus[2]

[1]Department of Paediatrics and [2]Department of Haematology, Addenbrookes Hospital, Hills Road, Cambridge CB2 2QQ, U.K.

High-dose chemotherapy followed by autologous bone marrow transplantation (ABMT) enables dose escalation in the treatment of childhood malignancies. Here we report our experience of using peripheral blood progenitor cells (PBPC) to restore haematopoiesis in five children using a simple cell mobilising regime and non-cryopreservation of the harvests. Cells were mobilised using cyclophosphamide and granulocyte colony stimulating factor. Each patient underwent only two leukapheresis, the product being stored before use at 4°C. Successful autologous PBPC transplantation was achieved with melphalan conditioning chemotherapy and re-infusion of the total progenitor cell product. No colony stimulating factors were administered after transplantation. The median numbers of mononuclear cells collected per patient was 10.0×10^8/kg (range 8.13–19.44) and CFU-GM 57.6×10^4/kg (range 10.4–178.85). All patients subsequently engrafted with the median number of days to a neutrophil count $>0.5 \times 10^9$/l being 11 (range 10–16), and to a platelet count $>50 \times 10^9$/l being 14 (range 12–31). The median number of in-patient days was only 20 (range 19–30). The median demand for blood was 2 units (range 1–2), and platelets 4 units (range 2–28). Usage of systemic antimicrobials and intravenous feeding was also low. Using this simple strategy, collection and transplantation of autologous progenitor cells can be a straightforward procedure in children. It is possible that this could enable dose escalation in some poor prognosis paediatric tumours. Copyright © 1996 Elsevier Science Ltd

Key words: ABMT, PBPC, melphalan, children, CFU-GM, mononuclear cell, cyclophosphamide, leukapheresis

Eur J Cancer, Vol. 32A, No. 11, pp. 1938–1942, 1996

INTRODUCTION

AUTOLOGOUS BONE marrow transplantation (ABMT) following high-dose chemotherapy is an established practice in the treatment of childhood cancers [1]. Recent interest has centred on the re-infusion of peripheral blood progenitor cells (PBPC) to restore haematopoiesis following high-dose therapy. The potential advantages of this technique include: reduced haematological recovery time and supportive care requirements [2, 3]; a significant reduction in cost [4]; avoidance of general anaesthesia for marrow harvesting; and the ability to autograft when conventional marrow harvesting is impossible due to pelvic radiotherapy.

Reports of successful haematopoietic reconstitution in children with PBPC first appeared in the late 1980s [5, 6]. Multiple leukaphereses were performed to coincide with the increased number of circulating progenitor cells previously observed during the recovery phase following chemotherapy. Subsequent studies utilised combinations of different drugs with colony stimulating factors to mobilise progenitor cells prior to collection [7, 8]. In an attempt to hasten engraftment colony stimulating factors were sometimes administered after transplantation [9]. In all cases, leukapheresis

Correspondence to N. Jones.
Received 11 Jan. 1996; revised 21 May 1996; accepted 23 May 1996.

Table 1. Patient details and previous treatment

Patient no.	Age (years)	Sex	Weight (kg)	Diagnosis	Status at time of transplant	Previous treatment
1	10	F	27.8	Rhabdomyosarcoma (stage 2)	Second CR	IVA VINCAEPI R/T Surgery
2	10	M	31.7	PNET (thoracic)	Second CR	OJEC IVA R/T Surgery
3	4	M	19.6	Neuroblastoma (stage 4)	First CR	OPEC/OJEC
4	4	F	13.2	Rhabdomyosarcoma (stage 4)	First CR	MMT Surgery
5	14	M	51.0	Medulloblastoma (bone metastases)	Second CR	Surgery R/T JOE

CR, complete remission; R/T, radiotherapy; MMT, ifosfamide, vincristine, actinomycin D, carboplatin, epirubicin, etoposide; VINCAEPI, vincristine, carboplatin, etoposide; OPEC, vincristine, cisplatin, etoposide, cyclophosphamide; OJEC, vincristine, carboplatin, etoposide, cyclophosphamide; JOE, carboplatin, vincristine, etoposide; IVA, ifosfamide, vincristine, actinomycin D; PNET, peripheral neuroectodermal tumour.

products were suspended in solutions of DMSO, frozen and thawed before re-infusion.

Here we report our experience using a simple progenitor cell mobilisation and storage regimen. Each patient underwent only two leukaphereses and the product was stored before use without cryopreservation. No colony stimulating factors were administered after transplantation. The time to haematopoietic recovery, duration of hospital stay and supportive care required are presented, demonstrating the advantages of this technique over conventional ABMT in children. This technique shows that complicated mobilising regimens, multiple harvests, cryopreservation and colony stimulating factors after transplantation may be unnecessary.

PATIENTS AND METHODS

Patients

Between June 1993 and February 1994, five children underwent therapy with high-dose melphalan and PBPC autografting. Patient details are shown in Table 1. Age ranged from 4 to 14 years and weight from 13.2 to 51.0 kg. At presentation, 2 patients (3 and 5) had bone marrow involvement, but at the time of PBPC harvesting all patients were in morphological bone marrow remission.

PBPC mobilisation

Prior to mobilisation, the patients had recovered from previous chemotherapy and had platelet counts $>100 \times 10^9$/l and leucocyte counts $>3 \times 10^9$/l. They received 30 mg/kg cyclophosphamide as an intravenous bolus and subcutaneous injections of 10 μg/kg GCSF on an out-patient basis begun the following day. This was continued until harvesting on the tenth and eleventh day of GCSF. Blood counts were monitored daily and harvesting began as the leucocyte count rose rapidly through 10×10^9/l (Figure 1).

PBPC collection

Using a Cobe Spectra Cell Separator programmed for mononuclear cell collection, two leukapheresis procedures were performed on consecutive days. The volume processed was 3× total blood volume with each collection lasting 3–4 h.

2 patients (2 and 5) had double lumen apheresis catheters inserted into the subclavian vein for harvesting and subsequent transplantation. The remainder already had internal jugular Broviac lines that were used to return blood to the patient and, on the day of the first harvest, a temporary percutaneous femoral dialysis catheter was inserted under ketamine sedation to serve as the outflow.

In view of the patients' relatively small calculated blood volume (900 ml in the youngest), CMV (cytomegalovirus) negative and irradiated donor red cells were used to prime the 180 ml of tubing in the cell separator. Acid citrate dextrose was used as an anticoagulant and serum calcium was

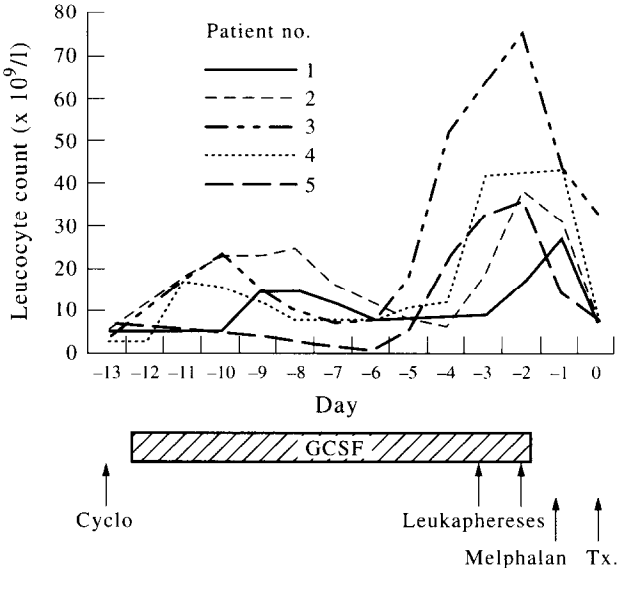

Figure 1. Timing of priming chemotherapy, leukaphereses, high-dose melphalan and progenitor cell transplantation. Cyclo, cyclophosphamide; Tx., PBPC transplantation.

Table 2. The volume, mononuclear cell (MNC) and CFU-GM content of each leukapheresis

	PBSC Harvest 1			PBSC Harvest 2			Total product infused		
Patient no.	Volume (ml)	MNC ($\times 10^8$/kg)	CFU-GM ($\times 10^4$/kg)	Volume (ml)	MNC ($\times 10^8$/kg)	CFU-GM ($\times 10^4$/kg)	Volume (ml)	MNC ($\times 10^8$/kg)	CFU-GM ($\times 10^4$/kg)
1	106	4.32	0.63	83	4.44	10.0	189	8.76	10.63
2	124	9.76	169.8	113	9.68	9.05	237	19.44	178.85
3	100	10.8	4.5	86	6.6	5.9	186	17.4	10.4
4	122	7.1	66	91	2.9	34.5	213	10.0	100.5
5	153	5.32	46.4	88	2.9	11.2	241	8.13	57.6

measured before, mid-way and post harvesting. No supplementary calcium was administered and none of the patients experienced symptoms of hypocalcaemia.

PBPC storage

The harvested cells in autologous plasma were anticoagulated with acid–citrate–dextrose (ACD) and stored at 4°C without agitation. Twenty-four hours after the melphalan the combined collections were infused, the first having been stored for 72 h and the second for 48 h.

CFU-GM assay

Granulocyte-macrophage progenitor cells (CFU-GM) were assayed in a semi-solid methyl cellulose culture system [10].

High-dose therapy and PBC transplantation

The patients were prehydrated with 4% dextrose/0.18% NaCl with 20 mmol/l KCl at 200 ml/m^2/h for 3 h. Melphalan 200 mg/m^2, as an intravenous bolus, was administered followed by 24 h posthydration using 3 l/m^2/24 h of 2.5% dextrose/0.45% NaCl with 40 mmol/m^2/24 h KCl.

The total PBPC collected was infused following the posthydration.

Supportive care post transplant

All the patients were nursed in single rooms on a general paediatric ward. Prophylactic antimicrobials were commenced on day 3 post-transplant (oral ciprofloxacin, penicillin V and fluconazole) and were continued until the neutrophil count reached 0.5×10^9/l. Thrice weekly septrin was then given for a total of 3 months.

Irradiated CMV negative blood product support was instituted to maintain a haemoglobin of >8 g/dl and a platelet count of $>10 \times 10^9$/l (20×10^9/l if infected or developing spontaneous petechiae).

Chlorhexidine mouthwashes were instituted from the day of transplant to maintain oral hygiene. Intravenous feeding was required in those patients unable to eat and drink sufficiently because of oral mucositis.

RESULTS

PBPC collection

Cyclophosphamide and GCSF were well tolerated with no adverse effects. There were no admissions for neutropoenic fever of blood products following the priming chemotherapy, although one patient developed transient neutropenia. Figure 1 demonstrates how PBPC collection started on the tenth day of GCSF as the leucocyte count rose rapidly through 10×10^9/l (median 33.0, range 9.4–52.0).

Transplantation and engraftment

The product of each harvest and the total number of cells re-infused is shown in Table 2. A median of 10.0×10^8/kg mononuclear cells were transplanted (range 8.13–19.44) corresponding to 57.6×10^4/kg CFU-GM (range 10.4–178.8). All patients engrafted. The median duration to a neutrophil count $>0.5 \times 10^9$/l was 11 days (range 10–16) and to a platelet count $>50 \times 10^9$/l was 14 days (range 12–31) (Table 3). Engraftment has so far been sustained throughout the period of follow-up (8–14 months).

Supportive care and infections (Table 3)

High-dose melphalan caused oral mucositis in all patients necessitating intravenous feeding in 3. 4 patients developed a pyrexia greater then 38°C and were treated with broad spectrum systemic antibiotics. All blood cultures were sterile, and the only obvious possible focus of infection in addition to oral mucositis was an area of erythema and induration at the site of femoral line insertion in patient 4. Intravenous amphotericin B was administered in 3 patients with persistent fever at 72 h, despite systemic antibiotics, and continued until resolution of fever and a neutrophil count of $> 0.5 \times 10^9$/l was attained.

Table 3. The number of in-patient days, supportive care requirements and time to neutrophil and platelet recovery

Patient no.	In-patient (days)	Blood (units)	Platelets (units)	TPN (days)	i.v. A (days)	i.v. AB (days)	Neutrophils >0.5 (days)	Platelets >50 (days)
1	30	1	28	22	14	2	13	31
2	23	2	2	10	7	0	11	12
3	20	1	3	0	0	3	16	14
4	20	2	5	9	8	4	10	22
5	19	2	4	0	4	0	11	13
Median	20	2	4	9	7	2	11	14

TPN, total parenteral nutrition; i.v. A, intravenous antibiotics; i.v. AB, intravenous amphotericin B.

Outcome

Patients 1 and 5 relapsed and died of disseminated disease at 14 and 6 months, respectively. However, there was no evidence of graft failure. Patient 2 is in remission having subsequently received radiotherapy for a new pleural deposit. All 3 survivors have normal peripheral blood counts at follow-up.

DISCUSSION

The earlier reports of successful PBPC autografting in children employed up to 10 leukaphereses in order to collect sufficient progenitor cells [6, 11]. As each run requires priming of the extracorporeal circulation with donor red cells, this presented a significant exposure to blood products with its inherent risks. Subsequent reports of increased progenitor cell mobilisation using a combination of myelosuppressive chemotherapy and GCSF obviated the need for so many leukaphereses. Complex regimens of drugs and GCSF, followed by as many as five harvests were still required to obtain median numbers of 22×10^4/kg CFU-GM and 4.5×10^8/kg mononuclear cells, respectively [9]. It has been suggested previously that successful haematopoietic reconstitution requires 10–30×10^4/kg CFU-GM [12]. Our experience suggests that a single dose of cyclophosphamide followed by 10 days of GCSF at 10 μg/kg enables a predictable rise in leucocyte count and collection of adequate numbers of PBPC to ensure haematopoietic recovery. With this regimen, we obtained a median of 57.6×10^4/kg (range 10.4–178.8) CFU-GM and 10.0×10^8/kg (range 8.13–19.44) mononuclear cells, which ensured adequate engraftment. The technique of multiple mobilisation runs and leukaphereses requires freezing of the products in a cryoprotectant such as dimethylsulphoxide (DMSO). Takaue and colleagues [11] found that after thawing, only 72% (±22%) of the initial number of cells prior to freezing remained viable. Adverse effects secondary to the DMSO infused were substantial. Of the 10 children transplanted, all suffered haemoglobinuria and vomiting, 5 required sedation or antihistamines for headache and flushing, 1 became confused and 2 experienced dyspnoea with cyanosis. Employing a similar mobilising regimen, Jestice and colleagues [13] demonstrated that progenitor cells committed to CFU-GM at the time of leukapheresis continue to divide when stored at 4°C, with CFU-GM numbers reaching their maximum at 48 h. At 72 h CFU-GM numbers have returned to their level at the time of collection and at 96 h 77% (±8%) of cells remain viable. This compares favourably with the numbers found following cryopreservation [11]. By transplanting PBPC stored at 4° for a maximum of 72 h, we have circumvented the hazards associated with DMSO, retained more viable cells and saved valuable resources.

Whether the PBPC mobilising technique could be further refined in order to increase the progenitor cell yield and perhaps obviate the need for 2 leukaphereses is not yet certain. To and colleagues [14] and Kotasek and colleagues [15] compared 4 g/m^2 and 7 g/m^2 of cyclophosphamide for PBPC mobilisation in adults with solid tumours. CFU-GM numbers were higher in those patients receiving 7 g/m^2, but the authors did not demonstrate a concomitant reduction in leukapheresis cycles or show that the subsequent PBPC transplants engrafted more rapidly or required less supportive care. Significant haematological and non-haematological morbidity were seen in both groups, with significantly longer periods of neutropenia and thrombocytopenia in the 7 g/m^2 group. Unfortunately, these studies did not employ GCSF in combination with cyclophosphamide, and it would be interesting to investigate the PBPC yield in such patients mobilised with different cyclophosphamide doses.

As the major cause of morbidity and mortality in patients undergoing autografting is the period of pancytopenia prior to engraftment, reducing this period would be of benefit. Comparison of haematological recovery times and supportive care requirements in patients treated by PBPC transplants, ABMT and allogeneic BMT demonstrates more rapid neutrophil and platelet recovery, reduced hospital stay, fewer febrile days and lower antibiotic, blood and platelet usage in those transplanted with PBPC [2–4]. We observed similar times to haematological reconstitution with neutrophil recovery at a median of 11 days (range 10–16) and platelets at 14 days (range 12–31). This resulted in a median duration of in-patient stay of only 20 days (range 19–30) with correspondingly small demands on supportive care (Table 3). With such a small number of patients transplanted, a statistical analysis of these results is not possible.

In an attempt to hasten engraftment in 7 children with solid tumours treated by high-dose melphalan and PBPC transplant, Fukuda and colleagues [9] continued to administer GCSF until haematological reconstitution. Median recovery times for neutrophils and platelets were 10 days (range 8–12) and 27 (range 14–73) suggesting the additional cost incurred was of no benefit.

In young children, it has been questioned whether there is anything to choose between a general anaesthetic for marrow harvest or the time spent on a cell separator, and that studies evaluating procedure costs are required [16]. We experienced no adverse effects from PBPC collection and 2–3 h spent in bed or on a chair seems preferable to a general anaesthetic and marrow harvest. At the time of leukapheresis, 3 of our patients (1, 3 and 4) already had internal jugular single lumen Broviac catheters which had been inserted during their preceding treatment protocols. In these patients, percutaneous femoral dialysis catheters were inserted under ketamine sedation to serve as the outflow. The remaining patients (2 and 5) had no central venous access on presentation for PBPC mobilisation so double lumen, tunnelled, apheresis catheters were inserted into the subclavian vein for harvesting and subsequent transplantation. Such catheters could be implanted into patients viewed as potential candidates for high-dose therapy and PBPC transplantation at the beginning of their respective treatment protocols, thus obviating the need for femoral dialysis catheters inserted under sedation at the time of leukapheresis.

Addressing the question of cost, Uyl-de Groot and associates compared PBPC transplants to conventional ABMT and ABMT augmented by GCSF. The total cost of the transplant decreased by more than 35% in the PBPC group [4].

We conclude that using a simple mobilising regime and non-cryopreservation of the product, collection and transplantation of autologous progenitor cells can be a straight-

forward procedure in children. Studies in adults have shown that PBPC re-infusion enables dose escalation [17], and it is possible that this strategy could improve the prognosis in poor risk paediatric tumours.

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