研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

Bone Marrow Transplantation (2006) 37, 583–587

www.nature.com/bmt

ORIGINAL ARTICLE

Preservation of ovarian function by ovarian shielding when undergoing total body irradiation for hematopoietic stem cell transplantation: a report of two successful cases

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The purpose of this study was to evaluate the possibility of preserving ovarian function by ovarian shielding to reduce the irradiation dose in total body irradiation (TBI). The subjects in the study were females aged less than 40 years, who were undergoing allogeneic hematopoietic stem cell transplantation using a TBI-based regimen and who desired to have children after transplantation. For ovarian shielding, abdominal computed tomography (CT) and skin marking were performed in both the supine and prone positions, prior to the TBI. A pair of columnar blocks was placed just above the patient's body. Thus far three patients have been treated. The serum estradiol level decreased to an undetectable level (<8.5 pg/ml) after transplantation and the follicle-stimulating hormone (FSH) level increased above 90 mIU/ml in all patients and they became amenorrheic. However, regular menstruation recovered in patients no. 1 and 2 about 800 and 370 days after transplantation, respectively, with a decrease in the serum FSH level. Menstruation did not recover in patient no. 3, and serum estradiol was transiently detected above 20 pg/ml. The preservation of ovarian function was made possible by ovarian shielding. However, a longer follow-up is needed to know if normal pregnancy and delivery can occur.

Bone Marrow Transplantation (2006) **37,** 583–587. doi:10.1038/sj.bmt.1705279; published online 30 January 2006

Keywords: ovarian function; total body irradiation; ovarian shielding;stem cell transplantation

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Received 16 June 2005; revised 21 November 2005; accepted 25 November 2005; published online 30 January 2006

Introduction

The conditioning regimen before allogeneic hematopoietic stem cell transplantation is intended to eradicate tumor cells and to promote immunosuppression to prevent graft rejection. A combination of cyclophosphamide and total body irradiation (TBI) is the most widely used regimen in transplantation for leukemia. However, this regimen causes severe germ cell injury and infertility.[1,2] On the other hand, patients who have received cyclophosphamide alone for aplastic anemia frequently recover ovarian function after transplantation. Considering that the dose of cyclophosphamide in transplantation for aplastic anemia is usually higher than that in transplantation for leukemia (200 vs 120 mg/kg), we explored the possibility of preserving ovarian function by reducing the irradiation dose by ovarian shielding.

Patients and methods

Patients

Three female patients aged less than 40 years, who were undergoing allogeneic hematopoietic stem cell transplantation using a TBI-based regimen and who desired to have children after transplantation, were the subjects of this study. The study was approved by the Ethics committee of the University of Tokyo Hospital and all patients gave informed consent to participate in this study.

Transplantation procedure

The preparative regimen was a combination of cyclophosphamide at 60 mg/kg/day for 2 days and TBI at 2 Gy twice daily for 3 days. In patient no. 3, the dose of cyclophosphamide was reduced to 40 mg/kg/day for 1 day and etoposide at 20 mg/kg/day for 2 days was added instead, because of impaired cardiac function before transplantation. Cyclosporin A was administered as a continuous infusion at a dose of 3 mg/kg/day combined with short-term methotrexate (10–15 mg/m^2 on day 1 and 7–10 mg/m^2 on days 3 and 6, and optionally on day 11) to prevent GVHD. Patient no. 3, who underwent transplantation from a

two-locus-mismatched sibling donor, received alemtuzumab at 0.2 mg/kg/day from day −8 to day −3.[13] Methylprednisolone at 1–2 mg/kg/day was added for patients who developed grade II–IV GVHD. Prophylaxis against bacterial, fungal and *Pneumocystis carinii* infection consisted of fluconazole, ciplofloxacin, and sulfamethoxazole/trimethoprim. For prophylaxis against herpes simplex virus infection, acyclovir was given 750 mg/day intravenously or 1000 mg/day orally from days −7 to 35, followed by long-term low-dose (400 mg/day) oral administration until the end of immunosuppressive therapy. A cytomegalovirus antigenemia assay using C10/C11 antibody was performed at least once a week after engraftment. Ganciclovir was started when more than two positive cells were detected on two slides.

TBI and ovarian shielding

Patients were treated in a mobile box made of 10 mm thick polymethyl methacrylate 600 mm wide by 2000 mm long by 400 mm high. The box is capable of moving up to 250 cm forward and backward on the rails with a constant speed. Beam intensity and moving velocity defined dose rate in TBI.[14] Normally, beam opening of the linac is $400 \times 10\,cm^2$. Leukemia patients were usually treated in the supine position for three fractions in the morning and in the prone position for three fractions in the evening.

The center of the mobile box was selected to be a reference point to attain the prescribed dose. Beam intensity and moving velocity were determined based on the measurement of the doses in Mix-DP slab phantoms with an ionization chamber, but no corrections for patient body size were required due to the use of the mobile box.

In TBI for the leukemia patients, most commonly, a pair of customized metal blocks was placed on the mobile box for lung shielding. The blocks were fabricated according to the lung shape, which was obtained by use of the X-ray film taken in the box. Lung shielding was performed in a fraction of TBI out of six fractions for three consecutive days in most cases.

For ovarian shielding, abdominal magnetic resonance imaging (MRI) and computed tomography (CT) were performed prior to the TBI. Position of the ovaries was checked with T2-weighted image of MRI and was projected and marked onto the patient's skin. Trans-abdominal ultrasound on the day of treatment was performed for the accurate positioning of the shields. As the ovarian shielding was performed in all six fractions, CT scan and skin marking were performed both in supine and prone positions. A pair of columnar blocks (8 cm in height and 5 cm in diameter) was placed just above the patient's body, as demonstrated in Figures 1 and 2. For ovarian shielding, beam opening was $40 \times 2\,cm^2$ to decrease penumbra. Figure 3 shows a portal image taken during an actual TBI with ovarian shielding.

Actual measurement for humanoid phantom

Actual doses to the ovary were measured with glass dosimeters within a humanoid phantom. Doses of 2 Gy in the supine position and 2 Gy in the prone position were given for total body with the tracking technique. Twelve glass dosimeters were placed at the ovarian position of the humanoid phantom under shielding (Figure 4).

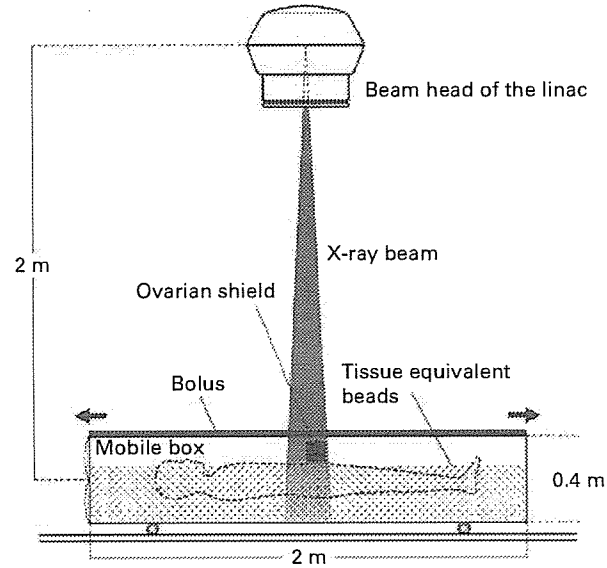

Figure 1 A schematic illustration of ovarian shielding in TBI.

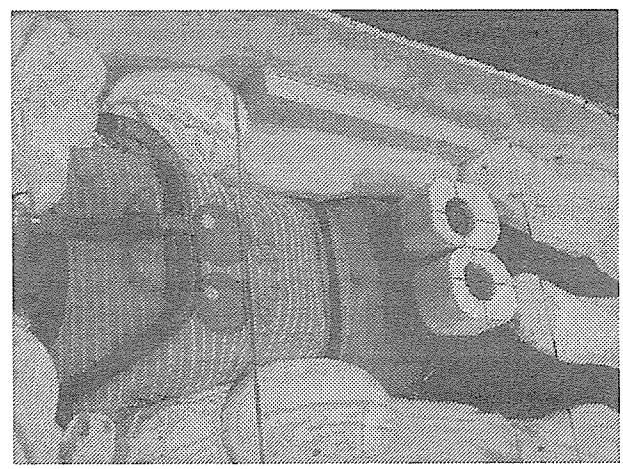

Figure 2 A pair of columnar blocks with dimensions of 8 cm in height and 5 cm in diameter. It was placed just above the patient's body.

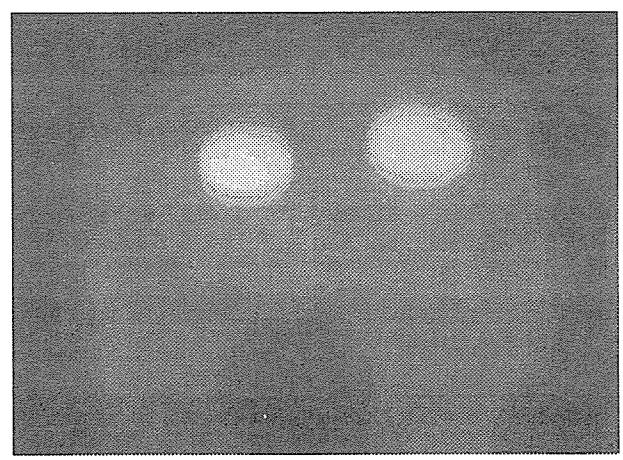

Figure 3 A portal image taken during an actual TBI with ovarian shielding.

Results

Patients

Thus far, three patients have been treated (Table 1). Two had chronic myelogenous leukemia in first chronic phase and had not received intravenous of antineoplastic agents before transplantation. The other patient had acute lymphoblastic leukemia in second remission and had received multiple courses of intensive chemotherapy. The donors were a matched unrelated donor, an HLA-identical sibling donor, and a two-locus-mismatched sibling donor in patients no. 1, 2, and 3, respectively. Patients no. 1 and 2 had regular menstruation before transplantation, but patient no. 3 already had chemotherapy-induced amenorrhea.

Transplantation outcome

All three patients had donor cell engraftment between days 15 and day 31 after transplantation. Acute GVHD was observed in only patient no. 1. She developed grade II acute GVHD limited to the skin, which was followed by extensive chronic GVHD. Patients no. 1 and 2 are alive without leukemia on days 1163 and 1055 after transplantation, respectively. However, patient no. 3 had a relapse of leukemia on day 223 and died on day 522.

Ovarian function after transplantation

The serum estradiol level decreased to an undetectable level (<8.5 pg/ml) after transplantation and the follicle-stimulating hormone (FSH) level increased above 90 mIU/ml in all patients and they became amenorrheic (Figure 5). However, patients no. 1 and 2 recovered regular menstruation about 800 and 370 days after transplantation, respectively, with a decrease in serum FSH level. In patient

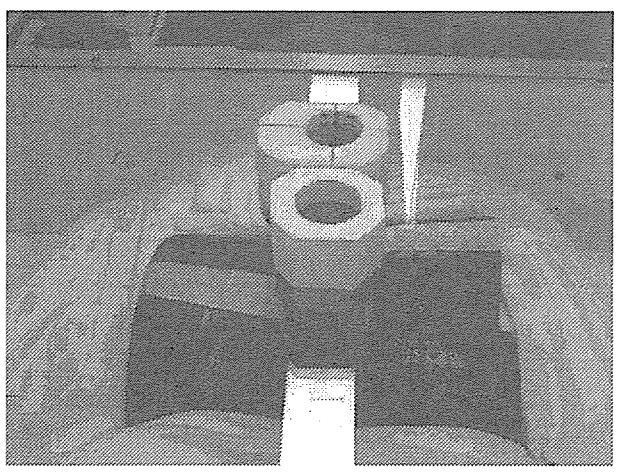

Figure 4 Humanoid phantom experiment.

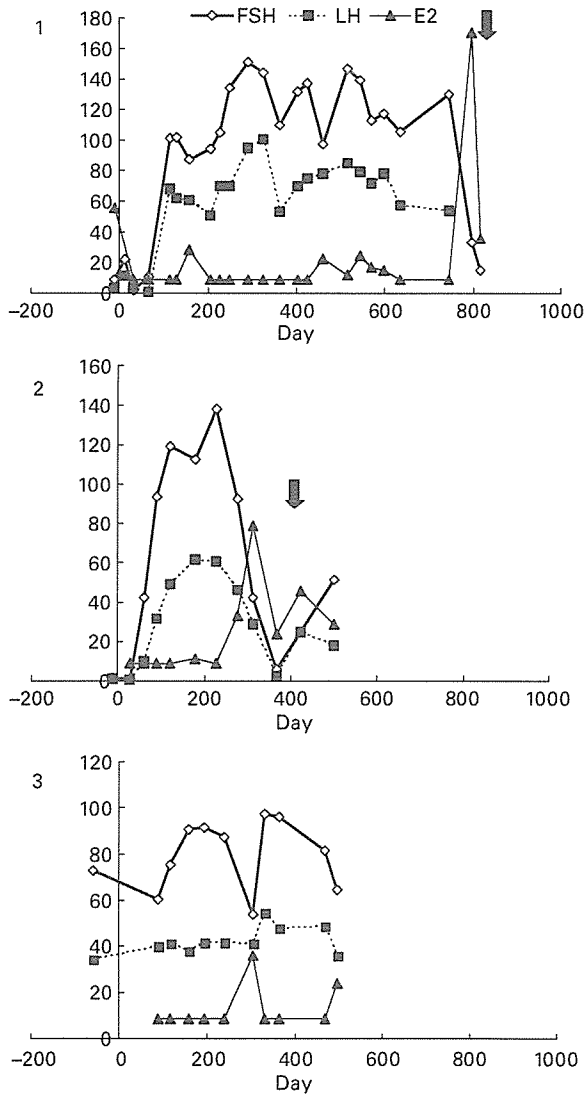

Figure 5 Ovarian function after transplantation. Arrows indicate the day of menstruation recovery.

Table 1 Patient characteristics

	Age[a]	*Diagnosis*	*Duration*[b]	*Prior Tx*	*Regimen*	*TBI dose (Gy)*	*Donor*
1	20	CML	7 years	HU, IFN	Cy/TBI	12	MUD
2	25	CML	6 months	HU	Cy/TBI	12	ISD
3	27	ALL	6 years	CCT	ETP/Cy/TBI	12	PMRD

[a]Age at transplantation.
[b]Duration form diagnosis to transplantation.
CML = chronic myelogenous leukemia; ALL = acute lymphoblastic leukemia; HU = hydroxyurea; IFN = interferon alpha; CCT = multiple courses of combined chemotherapy; Cy = cyclophosphamide; TBI = total body irradiation; ETP = etoposide; MUD = matched unrelated donor; MSD = HLA-identical sibling donor; PRMD = partially mismatched related donor.

no. 3, serum estradiol was transiently detected above 20 pg/ml, but she did not resume menstruating.

Assessment of basal body temperature and monitoring of follicle growth by sonohysterography would be useful to assess ovarian function of patients undergoing ovarian shielding in TBI.

Actual measurement for phantom

The mean and median actual doses measured by means of the glass dosimeters, which were inserted in the position of the ovaries in the humanoid phantom were between 1.041 and 1.042 Gy, respectively with a prescribed dose of 4 Gy. The range was 9.98–1.096 Gy. The results meant that the average total dose of the ovary was reduced from 12 to 3.123 Gy (74% less).

Discussion

The dose-limiting toxicity of TBI is interstitial pneumonia. Although the incidence of interstitial pneumonia has been significantly reduced by the use of fractionated irradiation compared to single dose irradiation,[13] 15% of patients still develop interstitial pneumonia after fractionated TBI. Therefore, lung shielding has been investigated to decrease lung toxicity of TBI. In a small nonrandomized study, the incidence of interstitial pneumonia was lower in patients who underwent TBI with lung shielding than in those who did not have shielding.[14] TBI may also affect renal function after transplantation. Therefore, Lawton *et al.*[15] attempted to protect renal function by renal shielding decreasing the total dose to the kidneys from 14 to 12 Gy, and the incidence of late renal dysfunction decreased from 26 to 6%.

The ovary is an organ sensitive to irradiation and the number of antral follicles per ovary has been shown to be reduced by ovarian irradiation in long-term survivors of childhood cancer.[16] Also, Shuck *et al.*[17] reported that all patients who received irradiation to the ovaries at greater than 15 Gy developed hormone failure. The radiation doses that cause 5 and 50% complications to the ovaries are about 3 and 10 Gy, respectively.[18] In this study, the irradiation dose to the ovaries was decreased by 75% by ovarian shielding and the total dose to the ovaries was estimated at about 3 Gy. Considering that recovery of ovarian function is frequently observed after a conditioning regimen of cyclophosphamide at 200 mg/kg only, the combination of cyclophosphamide at 120 mg/kg and TBI at 12 Gy with ovarian shielding should be reasonably protective to the ovaries.

Although patients who have received a conditioning regimen of cyclophosphamide and TBI may have spontaneous recovery of ovarian function long after transplantation, the incidence is less than 15% and it takes a median of 5 years for recovery of ovarian function after transplantation.[19] In this study, regular menstruation recovered in two of the three patients within 2 years after transplantation, showing the protective effect of ovarian shielding. However, spontaneous recovery of ovarian function is rarely seen after a combination of busulfan and cyclophosphamide, another major conditioning regimen for leukemia.[19 22] The risk of persistent alopecia is also more frequent after a busulfan-containing regimen.[23] Therefore, the combination of busulfan and cyclophosphamide should be avoided in young female patients, unless the patient has a condition that precludes the use of TBI, such as previous high-dose irradiation to a major organ.

It remains to be seen whether the recovery of ovarian function in these patients will allow a normal pregnancy and normal live birth. Recently, Carter *et al.*[24] analyzed pregnancy outcomes of female recipients and female partners of male recipients after hematopoietic stem cell transplantation. Seven females reported 13 pregnancies and 21 males reported 34 pregnancies. Most pregnancies were uncomplicated and resulted in 40 live births. Pregnancy outcomes were compared with those of their nearest-age siblings. The incidence of miscarriage or stillbirth was similar between the two groups. However, a larger study from the European Group for Blood and Marrow Transplantation[25] showed that the incidences of caesarean section, preterm deliverly, and low birthweight singleton birth offspring were higher compared to those in the normal population. Therefore, pregnancies in transplant recipients should be treated as high risks for maternal and fetal complications. In addition, the freezing of ovarian tissues or embryos might have a role as a back-up method of fertility treatment for the patient with ovarian failure after TBI.

We have shown that ovarian function could be preserved by ovarian shielding. However, a longer follow-up is needed to know whether this will allow normal pregnancy and delivery. Also needed is a larger study to evaluate the possible risk of increased relapse of leukemia after transplantation. In addition, the freezing of ovarian tissues or embryos might have a role as a back-up method of fertility preservation for patients who undergo hematopoietic stem cell transplantation and should be evaluated in the future.

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Biology of Blood and Marrow Transplantation 12:408-413 (2006)

1083-8791/06/1204-0003$32.00/0
doi:10.1016/j.bbmt.2005.11.007

A Randomized Controlled Trial to Compare Once- versus Twice-Daily Filgrastim for Mobilization of Peripheral Blood Stem Cells from Healthy Donors

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Received September 21, 2005; accepted November 7, 2005

ABSTRACT

Although the mobilization of peripheral blood stem cells from normal donors using granulocyte colony-stimulating factor is widely used, the ideal method for the administration of filgrastim has not been determined. Therefore, we compared the efficacy of peripheral blood stem cell mobilization on day 4 of filgrastim between once-daily (group O) and twice-daily (group T) administration of filgrastim at 400 μg/m^2/d. In all, 38 and 34 donors were randomly assigned to groups O and T, respectively. The number of CD34$^+$ cells collected on day 4 was not significantly different (1.74 × 10^6 cells/kg in group O and 2.08 × 10^6 cells/kg in group T, $P = .37$). The incidence and severity of adverse events were similar in the two groups. The baseline white blood cell count was the strongest predictor of poor mobilization. Donor age, sex, and serum concentrations of several cytokines did not significantly affect the CD34$^+$ cell yield. In conclusion, once-daily administration of filgrastim at 400 μg/m^2/d appeared to be appropriate for the mobilization of CD34$^+$ cells in normal donors when apheresis is planned on day 4 of filgrastim. Selection of a donor with a steady-state white blood cell count of 5.0 × 10^9/L or more may lead to a lower incidence of poor mobilization.

KEY WORDS

Peripheral blood stem cell transplantation • Mobilization • Granulocyte colony-stimulating factor • Filgrastim • Randomized controlled trial

INTRODUCTION

Peripheral blood stem cell (PBSC) graft is widely used for both autologous and allogeneic transplantation and produces faster neutrophil recovery than bone marrow (BM) cells [1]. A recent meta-analysis showed that allogeneic PBSC transplantation is associated with a decreased relapse rate and better survival in patients with late-stage hematologic malignancies compared with allogeneic BM transplantation, although it is also associated with a higher incidence of extensive chronic graft-versus-host disease [2]. As for donors, PBSC and BM donors experienced similar levels of discomfort, but the symptoms were resolved sooner in PBSC donors [3].

Granulocyte colony-stimulating factor (G-CSF) is the most widely used cytokine for PBSC mobilization.

However, the ideal method for PBSC mobilization has not been determined. A dose-response relationship between G-CSF and the number of collected $CD34^+$ cells has been shown in healthy donors and patients with cancer [4-7]. The European Group for Blood and Marrow Transplantation recommended the administration of G-CSF at 10 μg/kg/d for healthy PBSC donors [8]. Although most centers administer G-CSF once daily, others recommended the administration of G-CSF in two divided doses at a 12-hour interval [9,10]. In a retrospective study by Kröger et al. [10], more $CD34^+$ cells were collected with twice-daily (2 × 5 μg/kg/d) filgrastim than with once-daily (1 × 10 μg/kg/d) filgrastim. On the other hand, Anderlini et al. [11] showed that there was no difference in the efficacy of PBSC mobilization between the two methods. The only prospective randomized controlled trial showed that twice-daily administration was superior to once-daily administration in mobilizing $CD34^+$ cells on day 5 of G-CSF administration [12]. However, it is a common practice to start apheresis on day 4 to reduce the cost of G-CSF. Therefore, in this multicenter, open-labeled, randomized controlled trial, we intended to compare the efficacy of PBSC mobilization on day 4 of filgrastim, the frequency and severity of adverse events, and medical costs between once-daily and twice-daily administration of filgrastim. In addition, we prospectively evaluated the impact of potential confounding factors on $CD34^+$ cell yield, including serum levels of several cytokines.

DONORS AND METHOD

Donor Selection

Healthy PBSC donors aged 16 to 65 years were enrolled in this study. Donors with any organ dysfunction were excluded. This protocol was approved by each institutional review board and written informed consent was obtained from each donor.

Assignment, Mobilization, and Apheresis

Donors were randomly assigned to receive filgrastim either at 400 μg/m^2 once daily (group O) or at 200 μg/m^2 twice daily (group T). Assignment was stratified by the institute. Donors were hospitalized before filgrastim administration. Subcutaneous injection of filgrastim was started in the evening for group O, and in the morning for group T, to make the interval from the last filgrastim administration to the beginning of apheresis similar in both groups. The first apheresis was performed on the morning of day 4. When the number of collected $CD34^+$ cells per recipient body weight did not reach the target for each recipient, filgrastim was administered in the evening and apheresis was repeated on the next day. Apheresis was performed using a continuous-flow cell separator (COBE Spectra, Gambro BCT, Lakewood, USA). A total blood volume of 150 to 200 mL/kg was processed per apheresis at a flow rate of 60 to 80 mL/min.

Monitoring of Adverse Events

Physical examination, and subjective and objective findings were recorded at entry, every day from the start to the end of filgrastim administration, and at a follow-up a few weeks later. The severity of pain was recorded twice daily by the donors using the visual analog scale during filgrastim administration [13]. The severity of adverse events was recorded according to the National Cancer Institute-Common Toxicity Criteria version 2.0. For the treatment of bone pain caused by filgrastim, oral acetaminophen at 400 mg, with at least a 4-hour interval, was prescribed.

Statistical Analysis

The primary end point was the number of $CD34^+$ cells per donor body weight collected on day 4. Secondary end points included bone pain, the dose of acetaminophen, platelet counts immediately after the first apheresis, and medical costs. Poor mobilizers were defined as those with a collection of less than 2.0×10^6 $CD34^+$ cells per donor body weight on day 4. We planned to include 40 donors, 20 in each group, because 37 and 17 donors in each group were required to detect a difference in the number of $CD34^+$ cells of 1×10^6/kg and 1.5×10^6/kg, respectively, with a 2-tailed alpha error of 5% and a beta error of 20%. The target number of included patients was increased to 72 patients at an interim analysis. Continuous variables were analyzed with Student *t* test or the Mann-Whitney *U* test and dichotomous variables were analyzed with Fisher exact test.

RESULTS

Donors and Assignments

Between April 2001 and May 2002, a total of 72 healthy donors from 4 institutes were enrolled into this study. In all, 38 and 34 donors were assigned to the groups O and T, respectively. Filgrastim was not administered in one donor of group O because of the recipient's condition, and this donor was excluded from the analysis. The two groups were equivalent with regard to sex ($P = .64$), age ($P = .12$), body weight ($P = .70$), and white blood cell (WBC) ($P = .35$) and platelet ($P = .44$) counts. Group O included 22 male and 15 female members with a median age of 43 years (range 17-62). Group T included 18 men and 16 women with a median age of 51 years (range 19-65). The mean body weight of the donors was 60.1 kg (SD 10.7) in group O and 59.2 kg (SD 8.7) in group T, respectively. The mean WBC and platelet counts were 6.2 ×

10^9/L (SD 1.4) and 253 × 10^9/L (SD 56) in group O and 5.9 × 10^9/L (SD 1.1) and 243 × 10^9/L (S.D. 47) in group T, respectively.

Two donors in group O were erroneously given filgrastim at 200 μg/m^2 twice daily (protocol violation). These donors were included in group O in the intention-to-treat (ITT) analysis, but were included in group T in the per-protocol (PP) analysis. All of the donors completed a 3-day administration of filgrastim without any dose adjustment. Apheresis was performed once, twice, and three times in 27, 28, and 16 donors, respectively.

CD34$^+$ Cell Yield

As shown in Figure 1, the CD34$^+$ cell yields per donor body weight on day 4 fit a log normal distribution. Therefore, the statistical analyses for the CD34$^+$ cell yields were performed after a logarithmic transformation. The number of CD34$^+$ cells collected on day 4 was not significantly different in both ITT and PP analyses (Table 1). The geometric mean value was 1.74 × 10^6 cells/kg in group O and 2.08 × 10^6 cells/kg in group T (P = .37) in the ITT analysis and 1.77 × 10^6 cells/kg in group O and 2.02 × 10^6 cells/kg in group T (P = .51) in the PP analysis. A post hoc power analysis revealed that this study had the statistical power to detect a difference in the number of CD34$^+$ cells of 1.1 × 10^6/kg, with a 2-tailed alpha error of 5% and a beta error of 20%. Therefore, the lack of a significant difference between the two groups was not a result of a lack of adequate statistical power.

In the ITT analyses, there were 21 and 16 poor mobilizers among the 37 patients of group O and 34 of group T, respectively (57% versus 47%, P = .48). In the PP analyses, there were 19 and 18 poor mobilizers among the 35 patients of group O and 36 of group T, respectively (54% versus 50%, P = .81).

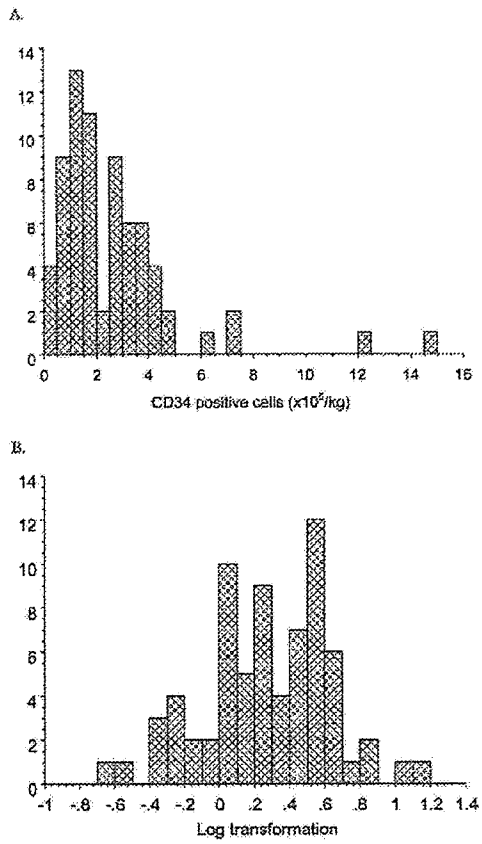

Figure 1. Number of CD34$^+$ cells per donor body weight (kg) collected on day 4 of G-CSF administration before (A) and after (B) logarithmic transformation.

Table 1. *Number of CD34$^+$ Cells Collected on Day 4 of Granulocyte Colony-Stimulating Factor Administration*

Intent to Treat No. of Subjects	Group O 37	Group T 34	Total 71
CD34$^+$ cell yield (×10^6/kg donor body weight)			
Geometric mean	1.74	2.08	
95% CI	1.30–2.32	1.57–2.76	P = .37
Per Protocol **No. of Subjects**	**Group O 35**	**Group T 36**	**Total 71**
CD34$^+$ cell yield (×10^6/kg donor body weight)			
Geometric mean	1.77	2.02	
95% CI	1.30–2.40	1.55–2.65	P = .51

CI indicates confidence interval.

Adverse Events and Medical Cost Analyses

Adverse events and medical costs were evaluated with PP analyses (Table 2). The area under the curve of the visual analog scale from day 1 to 4 was higher in group T, but this difference was not statistically significant (P = .15). The mean total dose of acetaminophen prescribed from day 1 to 4 was equivalent in the

Table 2. *Comparison of Adverse Events and Medical Costs Based on Per-Protocol Analysis*

No. of Subjects	Group O 35	Group T 36	Total 71
AUC of VAS (mm × ds)			
Median	21.5	51	
Range	0.0–195.5	0.0–230.0	P = .15
Total dose of acetaminophen (mg)			
Mean	729	783	
SD	916	900	P = .80
Postapheresis platelet count on day 4 (×10^9/L)			
Mean	124	114	
SD	160	146	P = .33
Duration of hospitalization			
Median	6	6	
Range	5–10	3–15	P = .87
Medical costs (¥)			
Mean	495,509	501,795	
SD	138,320	149,870	P = .85

AUC indicates area under the curve; VAS, visual analog scale.

Table 3. *Characteristics of 71 Patients Grouped According to the Outcome of Mobilization*

	Good Mobilizers	Poor Mobilizers	*P* Value
Factors before mobilization			
Age (y)	41.1 (12.7)	45.5 (14.4)	.18
Sex (male/female)	21/13	19/18	.47
Donor BW (kg)	60.6 (9.1)	58.7 (10.4)	.42
Group (O/T)	16/18	19/18	.81
WBC at entry ($\times 10^9$/L)	6.4 (1.2)	5.7 (1.2)	.02
Platelet at entry ($\times 10^9$/L)	250 (52)	247 (52)	.80
G-CSF (fmol/L)	527 (543)	553 (436)	.87
TPO (fmol/L)	4633 (1459)	5034 (3918)	.59
IL-3 (fmol/L)	39.4 (131)	215 (907)	.27
SCF (fmol/L)	44,654 (6402)	46,214 (8231)	.38
FLT-3L (fmol/L)	5504 (2613)	6005 (2335)	.41
EPO (fmol/L)	2856 (868)	3290 (1854)	.21
Factors on day 1 of G-CSF administration			
G-CSF C4 (pg/mL)	46,498 (28477)	45,920 (25364)	.94
Factors on day 4 of G-CSF administration			
WBC on day 4 ($\times 10^9$/L)	46.0 (9.6)	37.8 (9.3)	.0005
Platelet on day 4 ($\times 10^9$/L)	215 (51)	210 (48)	.71
$CD34^+$ on day 4 ($\times 10^9$/L)	0.056 (0.063)	0.016 (0.012)	.0004
Apheresis volume (L)	10.9 (1.87)	10.2 (1.3)	.10
Expression on the cell surface of harvested cells			
CD11a (%)	15.0 (7.9)	16.3 (6.6)	.58
CD49d (%)	16.8 (7.0)	17.3 (4.4)	.76
CD62L (%)	7.2 (3.2)	7.8 (3.5)	.55

BW indicates body weight; C4, plasma concentration 4 h after administration; EPO, erythropoietin; FLT-3L, Flt-3 ligand; G-CSF, granulocyte colony-stimulating factor; IL-3, interleukin-three; SCF, stem cell factor; TPO, thrombopoietin; O, once-daily; T, twice-daily (per-protocol analysis); WBC, white blood cell.

Poor mobilizers were defined as those with a collection of $<2.0 \times 10^6$ $CD34^+$ cells per donor body weight (kg) on day 4 of G-CSF administration. Numbers and those in parentheses show mean and SD, except for sex and group.

two groups (729 versus 783 mg, $P = .80$). The mean platelet count after the first apheresis was also similar in the two groups ($12.4 \times 10^4/\mu L$ versus $11.4 \times 10^4/\mu L$, $P = .33$). However, the platelet count after apheresis showed a significant negative correlation with donor age ($P = .03$). The median duration of hospitalization was 6 days in both groups ($P = .87$). The mean medical costs were 495,509 ¥ and 501,795 ¥ in groups O and T, respectively ($P = .85$).

Risk Factors for Poor Mobilization

We evaluated the impact of potential confounding factors on the $CD34^+$ cell yield (Table 3). Among the factors before mobilization, only the baseline WBC count was significantly different between the good and poor mobilizers (6.4×10^9/L versus 5.7×10^9/L, $P = .02$). As shown in Figure 2, there were no good mobilizers among donors with a baseline WBC count less than 5.0×10^9/L. Neither age nor sex significantly affected the $CD34^+$ cell yield (Figure 3). There were no difference in baseline serum concentrations of various cytokines between the two groups.

There was also no significant difference in the peak serum concentration of G-CSF ($P = .94$). As for factors on day 4, just before the first apheresis, both the WBC count and $CD34^+$ cells in the peripheral blood significantly correlated with the incidence of poor mobilization ($P = .0005$ and $P = .0004$, respectively). There was no difference in the expression of CD11a, CD49d, and CD62L on collected $CD34^+$ cells between good and poor mobilizers ($P = .58$, $P = .76$, and $P = .55$, respectively).

DISCUSSION

This randomized controlled trial demonstrated that once-daily administration of filgrastim at 400 $\mu g/m^2$ is as effective as twice-daily administration in two divided doses for the mobilization of $CD34^+$ cells in healthy donors. This result is different from that in the study by Kröger et al. [12], which showed that more $CD34^+$ cells were obtained by twice-daily administration than by once-daily administration (5.4×10^6 cells/kg versus 4.0×10^6 cells/kg, $P = .007$). The major difference between these two studies is the timing of apheresis. We evaluated the $CD34^+$ cell yield on day 4 of G-CSF administration, whereas Kröger et al. [12] performed their evaluation on day 5. Anderlini et al. [14] demonstrated in a sequentially comparative study that a single apheresis on day 5 yielded significantly more $CD34^+$ cells than that on day 4 ($P = .01$). However, it may still be reasonable to start apheresis on day 4 considering the cost of filgrastim, because about 40% of the donors could finish

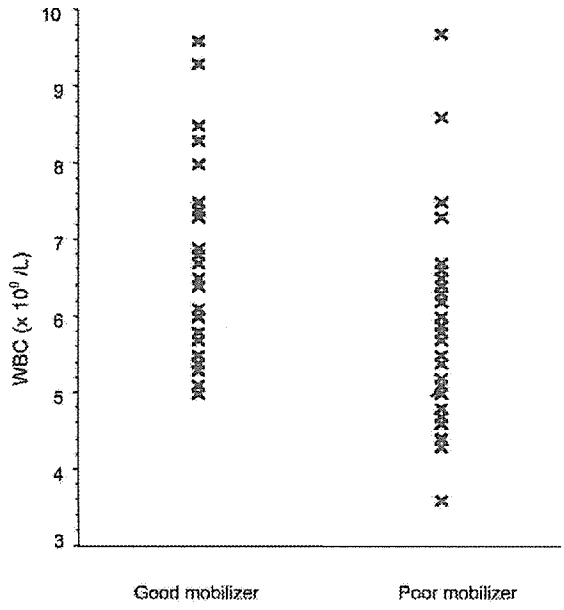

Figure 2. Peripheral blood WBC count before mobilization grouped according to outcome of mobilization. Poor mobilizers were defined as those with collection of less than 2.0×10^6 $CD34^+$ cells per donor body weight (kg) on day 4 of filgrastim administration.

apheresis on day 4. Both the number of collected $CD34^+$ cells and the incidence of poor mobilization on day 4 were equivalent between once-daily and twice-daily administration of filgrastim. Therefore, once-daily administration is generally recommended for PBSC mobilization from healthy donors, if the initiation of apheresis is planned on day 4. However, twice-daily administration may be better if a large number of $CD34^+$ cells is required, for example for haploidentical transplantation using $CD34^+$ cell selection, because apheresis on day 5 would be indispensable.

Several risk factors for poor mobilization have been retrospectively identified, including higher age and female sex [4,15-18]. In this study, the baseline low WBC count was the strongest predictor of poor mobilization. Especially, donors with a baseline WBC count of less than 5.0×10^9/L were exclusively poor mobilizers. Therefore, it may be advisable to select a donor with a high baseline WBC count if a patient has more than one healthy HLA antigen–matched donor. Surprisingly, donor age and sex had no impact on the yield of $CD34^+$ cells.

The steady-state plasma level of Flt-3 ligand showed a significant negative correlation with $CD34^+$ cell yield in patients undergoing high-dose chemotherapy and autologous stem cell rescue [19]. This may suggest that the plasma level of Flt-3 ligand is elevated in patients with poor BM function, because Flt-3 ligand induces multilineage hematopoietic cell differentiation and effectively mobilizes stem cells. Therefore, in this study, we evaluated the correlation between the $CD34^+$ cell yield and the serum levels of several hematopoietic cytokines including Flt-3 ligand, interleukin-3, stem cell factor, and G-CSF, but failed to find any significant correlations. Therefore, the measurement of steady-state serum cytokine levels is not useful for the prediction of poor mobilization in normal donors. The peak serum level of G-CSF also had no impact on the $CD34^+$ cell yield. The expression of adhesion molecules including L-selectin (CD62L), VLA-4 (CD49d/CD29), and LFA-1 (CD11a/CD18) may play an important role in stem cell mobilization and homing [20,21]. Therefore, we evaluated the expression of these adhesion molecules on collected $CD34^+$ cells. However, there was no difference in the expression levels of these molecules between good and poor mobilizers.

There were no differences in the incidence of adverse events between the two groups, although the area under the curve of visual analog scale tended to be slightly higher for group T. One of the major adverse events of concern is thrombocytopenia after apheresis. In this study, a significant negative correlation was observed between donor age and platelet count after the first apheresis. Although only one donor developed thrombocytopenia with less than 50×10^9/L after the first apheresis, we should carefully monitor the platelet count and bleeding tendency, especially in elderly donors.

In conclusion, once-daily administration of filgrastim at 400 $\mu g/m^2/d$ appeared to be appropriate for the mobilization of $CD34^+$ cells in normal donors when apheresis is planned on day 4 of filgrastim. Selection of a donor with a steady-state WBC count of 5.0×10^9/L or more may lead to a lower incidence of poor mobilization. Although an adequate number of $CD34^+$ cells can be collected from elderly donors, the careful monitoring of adverse events, especially the platelet count after apheresis, is important.

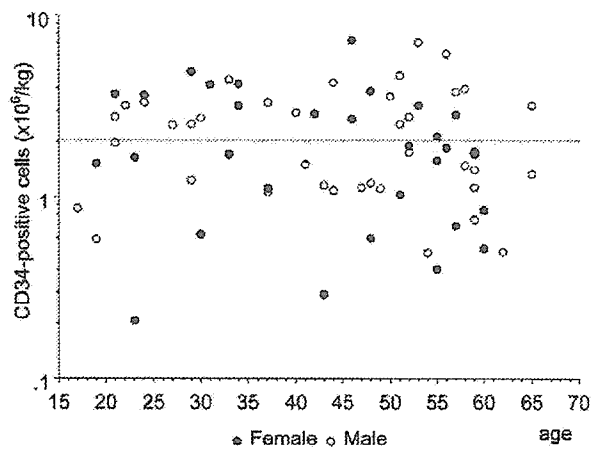

Figure 3. Correlation between age and number of $CD34^+$ cells per donor body weight (kg) collected on day 4 of G-CSF administration.

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Journal of Antimicrobial Chemotherapy (2006) **57**, 1004–1007
doi:10.1093/jac/dkl089
Advance Access publication 21 March 2006

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Pharmacokinetics of ganciclovir in haematopoietic stem cell transplantation recipients with or without renal impairment

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Received 3 October 2005; returned 22 November 2005; revised 25 January 2006; accepted 24 February 2006

***Objectives*: We investigated the pharmacokinetics of ganciclovir in 12 haematopoietic stem cell transplantation (HSCT) recipients to evaluate the validity of a 50% reduction in the ganciclovir dosage for mild renal impairment.**

***Patients and methods*: Ganciclovir at 5 mg/kg/day was pre-emptively infused in patients with estimated $CL_{CR} \geq 70$ mL/min (Group A), whereas the dose was reduced to 2.5 mg/kg/day in patients with CL_{CR} between 50 and 70 mL/min (Group B).**

***Results*: The peak concentration was significantly higher in Group A ($P < 0.01$). However, the decrease in the plasma ganciclovir concentration was slower in Group B ($P = 0.09$), and the AUC of all patients in both groups was distributed within a narrow range (25.6 ± 4.77 μg·h/mL), when two patients with exceptionally high AUC values were excluded.**

***Conclusions*: A 50% reduction in ganciclovir appeared to be appropriate for patients with mild renal impairment. Measuring the ganciclovir concentration at 4 h after starting infusion may be adequate for evaluating AUC.**

Keywords: cytomegalovirus, CMV, antigenaemia, antiviral therapy

Introduction

Ganciclovir is the mainstay of antiviral agents in pre-emptive therapy against cytomegalovirus (CMV) disease after allogeneic haematopoietic stem cell transplantation (HSCT).[1] Ganciclovir is mainly excreted from the kidney and about 90% of the administered dose is recovered unchanged in the urine after intravenous (iv) administration.[2] Therefore, total body clearance correlates well with CL_{CR}.[3,4] In HSCT settings, patients frequently develop renal impairment caused by the use of nephrotoxic drugs. A 50% reduction of ganciclovir is recommended in the drug information leaflet for patients with mild renal impairment of CL_{CR} between 50 and 70 mL/min in order to achieve an unchanged AUC. However, the pharmacokinetic profiles of ganciclovir have not yet been fully evaluated in such patients. Therefore, we investigated the validity of this dose reduction by serial evaluation of the plasma ganciclovir concentration.

Patients and methods

Twelve patients (nine men and three women) aged between 23 and 61 years were enrolled in a 12 h pharmacokinetic study of intravenous ganciclovir after ethical approval. The median age and weight were 50.5 years (range 23–61) and 57.5 kg (range 36.7–80.0), respectively. All patients provided informed consent to participate in this study. The underlying disease was acute leukaemia in three patients, chronic myelogenous leukaemia in three patients, myelodysplastic syndrome in two patients and pancreatic cancer in four patients. Five patients received a graft from an HLA-matched relative and seven received a graft from an alternative donor defined as an HLA-mismatched relative or a matched unrelated donor. We calculated CL_{CR} weekly, based on a 24 h urine collection. Patients were classified into two groups according to CL_{CR} evaluated within 1 week before the initiation of ganciclovir administration: Group A included seven patients with $CL_{CR} \geq 70$ mL/min (mean 98.1 mL/min, range 74.9–142.0 mL/min) and Group B included five patients

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Table 1. Pharmacokinetic parameters of ganciclovir in Groups A and B

	Group A (CL_{CR} ≥ 70 mL/min)	Group B (CL_{CR} 50–70 mL/min)	*P* value
C0.5	6.56 (4.39–11.33) μg/mL	4.92 (2.90–10.80) μg/mL	0.37
C1	9.20 (5.50–19.03) μg/mL	4.75 (3.32–6.61) μg/mL	<0.01
C2	4.76 (2.72–12.09) μg/mL	2.38 (2.30–2.73) μg/mL	<0.01
C4	2.58 (1.25–6.30) μg/mL	1.57 (1.37–1.80) μg/mL	0.17
C6	1.69 (0.79–4.89) μg/mL	1.15 (0.90–1.30) μg/mL	0.29
C8	1.22 (0.40–3.99) μg/mL	0.91 (0.64–1.09) μg/mL	0.57
C12	0.62 (0.23–2.88) μg/mL	0.58 (0.39–0.81) μg/mL	0.94
LogC4/C1	–0.66 (–0.73– –0.48)	–0.42 (–0.68– –0.33)	0.09
AUC	29.8 (20.2–111.0) μg·h/mL	24.6 (22.5–28.3) μg·h/mL	0.57
$t_{1/2}$	3.57 (3.36–7.94) h	5.76 (5.05–8.87) h	0.03
CL_{TOT}	3.04 (0.73–4.31) mL/min/kg	1.66 (1.50–1.81) mL/min/kg	0.12

CL_{CR}, creatinine clearance; AUC, area under the concentration curve; $t_{1/2}$, elimination half-life; CL_{TOT}, total body clearance.
C0.5–C12 represent plasma ganciclovir concentrations at 30 min, and 1, 2, 4, 6, 8 and 12 h after start of infusion, respectively.
The values of each parameter are reported as the median and range.

with CL_{CR} between 50 and 70 mL/min (mean 59.1 mL/min, range 51.3–67.4 mL/min).

Antigenaemia assay for CMV infection was performed weekly after engraftment as described previously.[5] Ganciclovir was pre-emptively started when 20 or more positive cells were detected per two slides in patients who received a graft from an HLA-matched relative, whereas it was started when three or more positive cells were detected per two slides in patients who received a graft from an alternative donor. The starting dose of ganciclovir was once daily at 5 and 2.5 mg/kg/day in Groups A and B, respectively, which was infused at a constant rate over 1 h.[6] Venous blood samples were obtained before infusion (C0), 30 min (C0.5) and 1 (C1), 2 (C2), 4 (C4), 6 (C6), 8 (C8) and 12 (C12) h after starting the first-dose infusion. After the blood sample was centrifuged, the plasma was separated and stored at –20°C until measurement of the ganciclovir concentration.

The plasma ganciclovir concentration was measured after solid-phase extraction (SPE) and dilution in mobile phase by reversed-phase HPLC. In brief, plasma samples were heated at 58°C for 30 min to inactivate the virus prior to handling. These samples were then diluted with 0.1 M phosphate buffer (pH 8.0) and applied to disposable C_{18} SPE columns (Bond Elut C18-OH; Varian, Palo Alto, CA, USA) conditioned with methanol and water. The column was washed with 0.1 M phosphate buffer (pH 8.0) and water, and ganciclovir was then eluted by 1.5 mL of 15% methanol. After 0.1 mL of 10 μg/mL guanosine was added as an internal standard, the eluent was injected into the HPLC system (C_{18} column, CAPELL PAK C18 SG 120; Shiseido, Tokyo, Japan; mobile phase: a mixture of 20 mM KH_2PO_4 (pH 2.6) containing 5 mM sodium 1-octanesulfonate and acetonitrile (95 : 5, v/v)]. The flow rate of the mobile phase and the column temperature were 0.8 mL/min and 40°C, respectively. The HPLC was equipped with a photo diode array detector (SPD-M10A vp, Shimadzu, Kyoto, Japan) set at a detection wavelength of 254 nm. This quantitative assay provided a high selectivity for determining a compound in biological samples. It was available for 0.02–5 μg/mL of an analyte in plasma samples. The precision expressed as a coefficient of variation was less than 2.5%, and the accuracy expressed as an error per cent was <±3%. Endogenous sources of interference were not detected from blank plasma.

Pharmacokinetic parameters were calculated by non-compartment modelling using WinNonlin software (version 4.0; Pharsight Corporation). CL_{CR} was normalized to 1.73 m^2 body surface area and AUC was calculated using the linear trapezoidal rules with extrapolation to infinity by standard techniques. The decline ratio was calculated as Log C4/C1 for the evaluation of the decrease in plasma ganciclovir concentration in the distribution phase and early elimination phase, whereas the elimination half-life was calculated from the terminal portion of the slope after C4. The differences between groups were compared using the Wilcoxon (Mann–Whitney)-test. *P* values of less than 0.05 were considered statistically significant. The relationship between the total AUC and plasma ganciclovir concentration at each point after starting infusion was investigated by calculating correlation coefficients r^2 using linear regression analysis after logarithmic transformation because they did not fit a normal distribution.

Results

The median pharmacokinetic parameters and the concentration versus time profile are shown in Table 1 and Figure 1(a). The peak plasma concentration (C_{max}) ranged from 3.32 to 19.03 μg/mL. The C_{max} in Group A was significantly higher than that in Group B (9.20 versus 4.75 μg/mL, $P < 0.01$). There was a borderline significance in the decline ratio between the two groups (–0.66 versus –0.42, $P = 0.09$). Total body clearance in Group B was lower than that in Group A (1.66 versus 3.04 mL/min/kg, $P = 0.12$). Also, the elimination half-life in Group B was significantly longer than that in Group A (5.76 versus 3.57 h, $P = 0.03$). There was no significant difference in AUC between the two groups (29.8 versus 24.6 μg·h/mL, $P = 0.57$). The AUCs of the patients in both groups were distributed within a narrow range (25.6 ± 4.77 μg·h/mL, Figure 1b), when we excluded two patients with exceptionally high AUC values (48.18 and 110.99 μg·h/mL). The CL_{CR} values of these two patients were 74.9 and 87.2 mL/min, respectively. Among the serial ganciclovir concentration measurements, C4 most strongly correlated with AUC ($r^2 = 0.95$, Figure 1c).

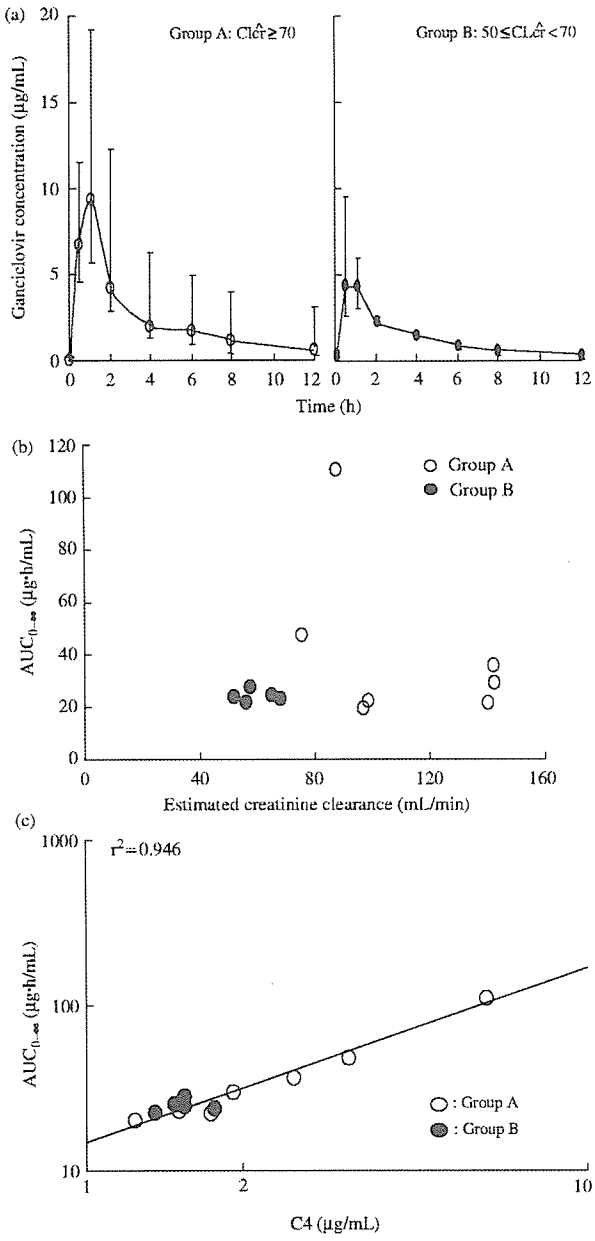

Figure 1. (a) Median concentrations of ganciclovir after 1 h iv infusion of 5 mg/kg ganciclovir in Group A and of 2.5 mg/kg ganciclovir in Group B. Open and filled circles represent each median concentration point in Groups A and B, respectively. (b) The AUC in each patient. Open and filled circles represent individual measurements in Groups A and B, respectively. (c) Correlation between the AUC and the plasma concentration at 4 h after starting infusion (C4). Open and filled circles represent individual measurements in Groups A and B, respectively. The solid line represents the orthogonal regression line described by the equation AUC = 17.666 × C4 – 4.4555.

Discussion

The results demonstrated that a 50% reduction in the ganciclovir dosage was appropriate for HSCT recipients with mild renal impairment of CL_{CR} between 50 and 70 mL/min. In addition to the significant difference in the elimination half-life, we observed a difference in the decline ratio (Log C4/C1) between the two groups with a borderline significance, which might indicate that renal excretion had started within 4 h of infusion. AUC was not significantly different from that in patients with normal renal function, probably due to the prolonged elimination in patients with mild renal impairment, although the small sample size might be responsible for the lack of significant difference. When we excluded two patients whose AUC values were exceptionally high, the AUC ranged within 25.6 ± 4.77 μg·h/mL, which was similar to the values reported previously.[4] An exceptionally high AUC was observed in two patients with CL_{CR} values between 70 and 90 mL/min. The reason for the high AUC is not clear, but it may suggest that the dose of ganciclovir should be reduced in patients with CL_{CR} values between 70 and 90 mL/min after confirming that the AUC is significantly high in such patients. Drug interaction is also a possible explanation for the high AUC, but these two patients were not being given drugs that are known to interact with ganciclovir. Also, the exceptionally high AUC might result from a transient renal dysfunction, which could not be detected even by a weekly CL_{CR} examination.

The role of clinical pharmacokinetic monitoring in solid organ transplantation as well as in HSCT is unclear.[7] Previous studies failed to show a significant correlation between the ganciclovir concentration and its efficacy or toxicity.[7,8] A possible explanation for this lack of correlation is the small number of patients in these studies, since a significant correlation between the cumulative dose of ganciclovir and the incidence of neutropenia has been shown in large-scale clinical studies.[9,10] However, it is difficult to perform a large-scale study with pharmacokinetic monitoring because of the need for repeated blood sampling from patients. In this study, C4 most strongly correlated with AUC, with r^2 values of 0.95, although we should confirm this in a larger study. Another limitation of pharmacokinetic monitoring of ganciclovir is that only the intracellular phosphorylated ganciclovir is active and it is not known how its concentration relates to the plasma concentrations. Nevertheless, a prospective study with monitoring of C4 is warranted to evaluate the role of pharmacokinetic monitoring in HSCT.

In conclusion, a recommended reduction of ganciclovir dosage by 50% appeared to be appropriate for HSCT recipients with mild renal impairment. Measurement of the plasma ganciclovir concentration C4 could be an accurate predictor of AUC. Further studies are necessary to validate these findings in a larger number of patients and to clarify the relationship among plasma concentrations, AUC and responses.

Transparency declarations

None to declare.

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American Journal of Hematology 81:875–879 (2006)

Pharmacokinetics of Alemtuzumab after Haploidentical HLA-Mismatched Hematopoietic Stem Cell Transplantation Using in Vivo Alemtuzumab With or Without CD52-Positive Malignancies

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We recently reported that the addition of in vivo alemtuzumab to the conditioning regimen enables 2- or 3-locus-mismatched hematopoietic stem cell transplantation without an excessive risk of graft rejection or graft-versus-host disease. In a later series of patients, however, one patient with refractory chronic lymphocytic leukemia with large residual tumors at transplantation developed graft rejection. While the peak alemtuzumab concentration in the previous patients without graft rejection was higher than 5 μg/ml, the peak alemtuzumab concentration in this patient was only 1.44 μg/ml. We considered that alemtuzumab was bound to the large residual tumors, which resulted in a low blood concentration of alemtuzumab. Therefore, it is important to debulk tumors before the conditioning regimen for patients with refractory CD52-positive hematological malignancies, or the dose of alemtuzumab should be adjusted by monitoring the blood concentration, when alemtuzumab is used for in vivo T-cell depletion in 2- or 3-locus-mismatched transplantation. Am. J. Hematol. 81:875–879, 2006.

Key words: chronic lymphocytic leukemia; hematopoietic stem cell transplantation; alemtuzumab; serum concentration; rejection

INTRODUCTION

Alemtuzumab (Campath-1H) is a humanized monoclonal antibody directed against human CD52 that is expressed at a high density on B- and T-cells and dendritic cells, but not on hematopoietic stem cells [1]. Although alemtuzumab was approved for the treatment of fludarabine-refractory chronic lymphocytic leukemia (CLL) [2], it has also been used for in vivo T-cell depletion to prevent graft rejection and graft-versus-host disease (GVHD) in allogeneic hematopoietic stem cell transplantation (HSCT) [3,4]. The addition of alemtuzumab to a conditioning regimen decreases graft rejection by depleting host T-cells. In addition, it has a long terminal half-life (15–21 days) and the blood concentration is maintained at a lympholytic level for about 2 months after transplantation, which contributes to the prevention of GVHD [5]. We extended the use of in vivo alemtuzumab to 2- or 3-locus-mismatched transplantation and successfully reduced the incidence of grade III-IV acute GHVD to only 9% without graft rejection in the first 12 patients in a prospective study approved by the ethics commitee [6]. However, in a later series of patients, a patient with fludarabine-refractory CLL with large residual tumors at transplantation developed graft rejection after an initial neutrophil recovery. We describe here the clinical course and discuss the pharmacokinetics of alemtuzumab in patients with or without CD52-positive hematological malignancies.

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Received for publication 3 October 2005; Accepted 18 March 2006

Published online 21 July 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20694

Clinical Course of a Patient Who Developed Graft Rejection

A 56-year-old woman with CLL, which was refractory to 10 courses of fludarabine (30 mg/m^2 × 5 days), 2 courses of rituximab (375 mg/m^2), and 4 courses of CVP therapy (cyclophosphamide 750 mg/m^2 × 1 day, vincristine 1.4 mg/m^2 × 1 day, prednisolone 60 mg/m^2 × 5 days), chose to participate in a clinical study of 2- or 3-locus-mismatched HSCT using in vivo alemtuzumab, since she did not have an available HLA-matched or 1-locus-mismatched donor among her family members and her disease status precluded a time-consuming donor coordination to identify an HLA-matched unrelated donor. Just before starting the conditioning regimen, she still had large residual tumors in the abdomen, although the peripheral blood lymphocyte count was decreased to 2.66 × 10^9/L. The conditioning regimen consisted of alemtuzumab (0.2 mg/kg/day from day −8 to −3), fludarabine (30 mg/m^2 from day −8 to −3), busulfan (4 mg/kg/day on days −5 and −4) and total body irradiation (TBI; 2 Gy twice daily on day −1). Peripheral blood mononuclear cells were collected from her 3-locus-mismatched son following a mobilization with filgrastim, cryopreserved without ex vivo manipulation, and infused on day 0. The number of infused CD34- and CD3-positive cells was 4.75 × 10^6 cells/kg and 0.86 × 10^8 cells/kg of recipient body weight, respectively. Posttransplantation prophylaxis against GVHD was performed with the continuous infusion of cyclosporine A (3 mg/kg) and short-term methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11). Regimen-related toxicities were mild. Neutrophil engraftment, defined as the first of 3 consecutive days with an absolute neutrophil count of at least 0.5 × 10^9/L, was documented on day 15. On day 18, however, the granulocytic count began to rapidly decrease, associated with a high fever up to 104°F, disseminated intravascular coagulation, and a high lactate dehydrogenase level. We restarted filgrastim but the neutrophil count decreased to below 0.10 × 10^9/L. Eighty-two percent of the bone marrow cells were of donor origin on day 22, but donor cells became undetectable in both the bone marrow and peripheral blood on day 28. The abdominal CT scan on day 22 showed decreased but residual tumors. Flow cytometry analysis of the peripheral blood on day 26 showed that more than 90% of lymphocytes were CD8-positive T-cells. Although we waited for autologous hematopoietic recovery, the neutrophil count remained below 0.10 × 10^9/L. Therefore, we performed the second peripheral blood stem cell transplantation from the same donor on day 51 after the first transplantation following a conditioning regimen consisting of alemtuzumab (0.2 mg/kg/day from day −10 to −5), cyclophosphamide (30 mg/kg/day on days −7 and −6), and fludarabine (25 mg/m^2/day from day −5 to −1). The number of CD34- and CD3-positive cells infused at the second transplantation was 2.86 × 10^6 cells/kg and 0.53 × 10^8 cells/kg of recipient body weight, respectively. We started the continuous infusion of CsA on day −1. However, she developed acute renal failure and thus we replaced CsA with prednisolone at 1 mg/kg/day from day 2. At the same time, high fever, skin rash, fluid retention, weight gain, and noncardiogenic pulmonary edema rapidly progressed, which required mechanical ventilation from day 2 and continuous hemodiafiltration from day 9. Twice, we administered high-dose methyl-prednisolone at 1000 mg/day for 3 days (from days 9 and 15). Despite these treatments, capillary leak syndrome did not improve and she died on day 18 due to severe hypotension, although donor cell engraftment was confirmed on day 11. The clinical course of the CLL patient is summarized in Figure 1. Autopsy revealed no residual CLL cells and no microbiologically documented infections. Pathological finding of the skin showed the degeneration of epidermal cells and sweat gland cells with little lymphocyte infiltration, which were compatible with acute GVHD.

Blood Concentration of Alemtuzumab

The serum concentration of alemtuzumab was determined by indirect immunofluorescence using frozen sera as described in detail elsewhere [7,8]. The serial serum concentrations of alemtuzumab of the present patient in the first and second transplantations and those of three control patients who underwent haploidentical HSCT using alemtuzumab from a 3-locus-mismatched related donor and whose serum samples before and after transplantation were available are shown in Figure 2. The current patient and the three control patients participated in the same study to evaluate the safety of unmanipulated peripheral blood stem cell transplantation from 2- or 3-locus-mismatched related donors using alemtuzumab in vivo and received exactly the same alemtuzumab dosage schedule (0.2 mg/kg/day from day −8 to −3) and the same supportive care [6]. Of the three control patients, two (C1 and C2) had myeloid malignancies and the other (C3) had diffuse large B-cell lymphoma in partial remission. Patients C2 and C3 received the same conditioning regimen as the CLL patient did, whereas patient C1 received TBI at 2 Gy twice daily on days −7, −6, and −5, followed by cyclophosphamide at 60 mg/kg on days

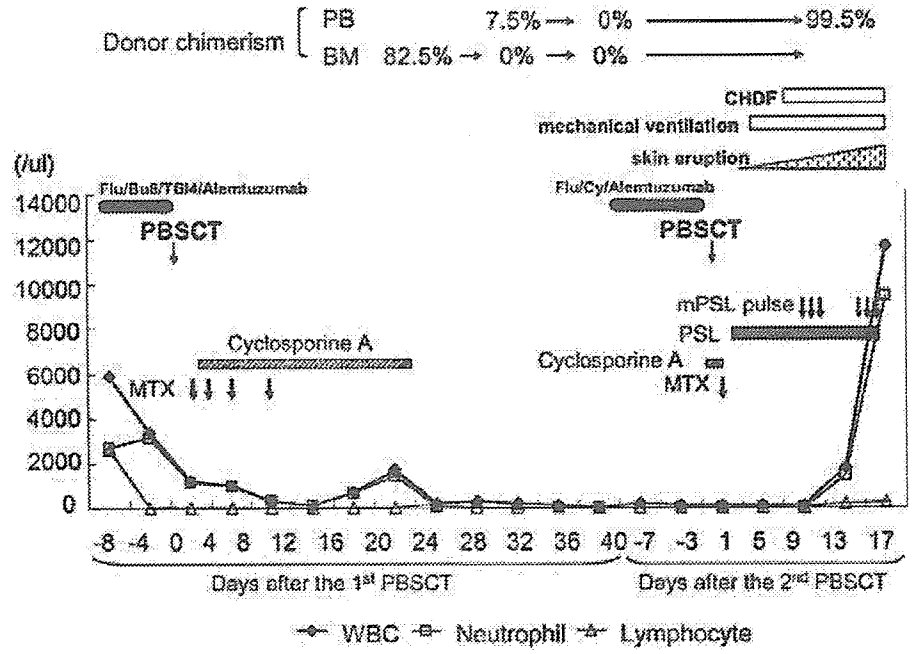

Fig. 1. Clinical course of the first and second haploidentical transplantation using in vivo alemtuzumab. PB, peripheral blood; BM, bone marrow; CHDF, continuous hemodiafiltration; Flu, fludarabine; Bu, busulfan; TBI, total body irradiation; Cy, cyclophosphamide; PBSCT, peripheral blood stem cell transplantation; MTX, methotrexate; PSL, prednisolone; WBC, white blood cell.

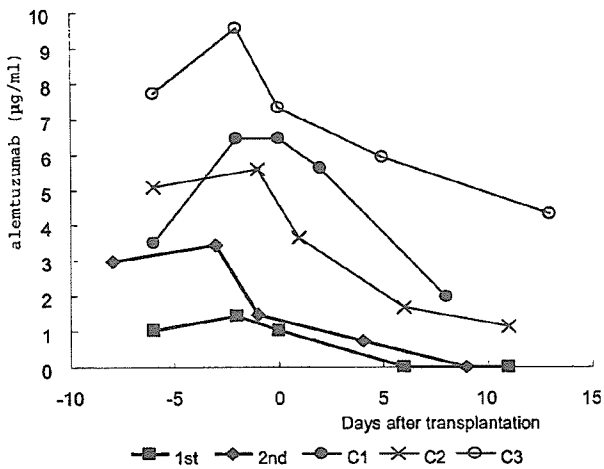

Fig. 2. Serial serum concentrations of alemtuzumab. Alemtuzumab activity was measured by indirect immunofluorescence. The lower limit of this assay for serum samples is 0.50 μg/ml and the upper limit is 20.00 μg/mL.

−3 and −2. Although the serum alemtuzumab concentrations in the three control patients were comparable to those in previous studies where alemtuzumab was used in a conditioning regimen [9,10], the serum alemtuzumab concentration in the CLL patient was persistently lower than 2.0 and 4.0 μg/ml in the first and second transplantations, respectively, and it quickly decreased to an undetectable level (<0.5 μg/ml) after transplantation. Therefore, the serum concentration of alemtuzumab before transplantation was too low to suppress host T-cells, which may have resulted in graft rejection after the first transplantation. In the second transplantation, host T-cells might have been sufficiently suppressed by the repeated conditioning regimen, but a strong reaction compatible with hyperacute GVHD occurred a few days after the infusion of donor graft, probably due to the insufficient alemtuzumab concentration on day 0 and thereafter.

DISCUSSION

The use of in vivo alemtuzumab in an HSCT setting enables durable engraftment and a significant reduction of GVHD, even in 2- or 3-locus-mismatched HSCT [6,10]. Pharmacokinetic studies of alemtuzumab at a dose of 20 mg/day for 5 days before transplantation with a reduced-intensity conditioning regimen have demonstrated that the serum alemtuzumab concentration was higher than the level that was required to kill infused donor T-cells at the time of transplantation and remained at