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8. **知的所有権の出願・取得状況(予定を含む)**

該当無し

Ⅱ．研究成果の刊行に関する一覧

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Ⅲ. 研究成果の刊行物・別刷

Bone Marrow Transplantation (2006) 37, 583–587

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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.

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

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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 12] 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).

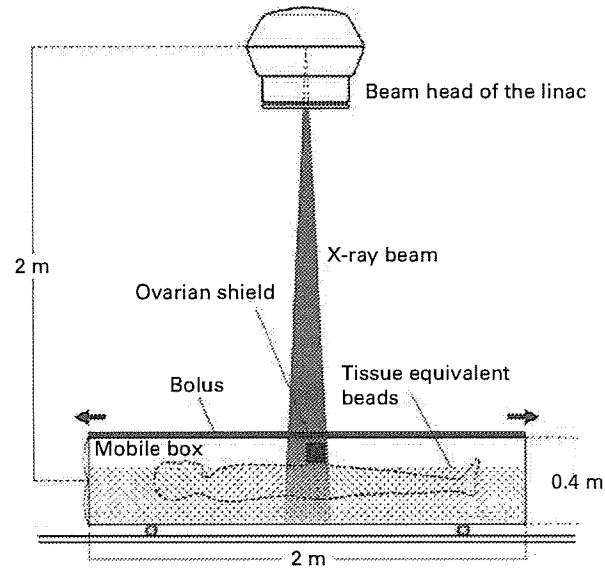

Figure 1 A schematic illustration of ovarian shielding in TBI.

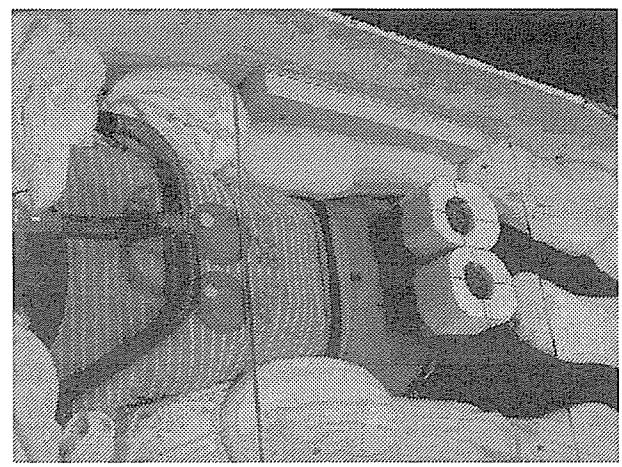

Figure 2 A pair of columnar blocks with dimensions of 8 cm in height and 5 cm in diameter. It was placed just above the patient's body.

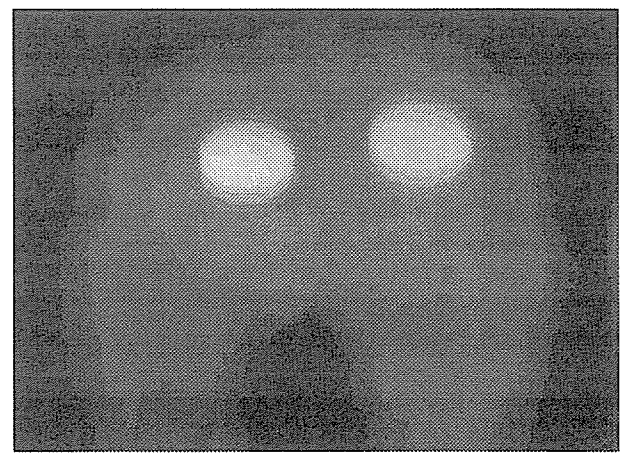

Figure 3 A portal image taken during an actual TBI with ovarian shielding.

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

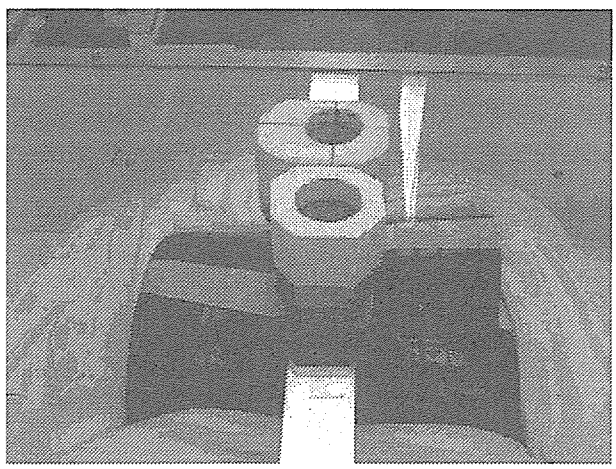

Figure 4 Humanoid phantom experiment.

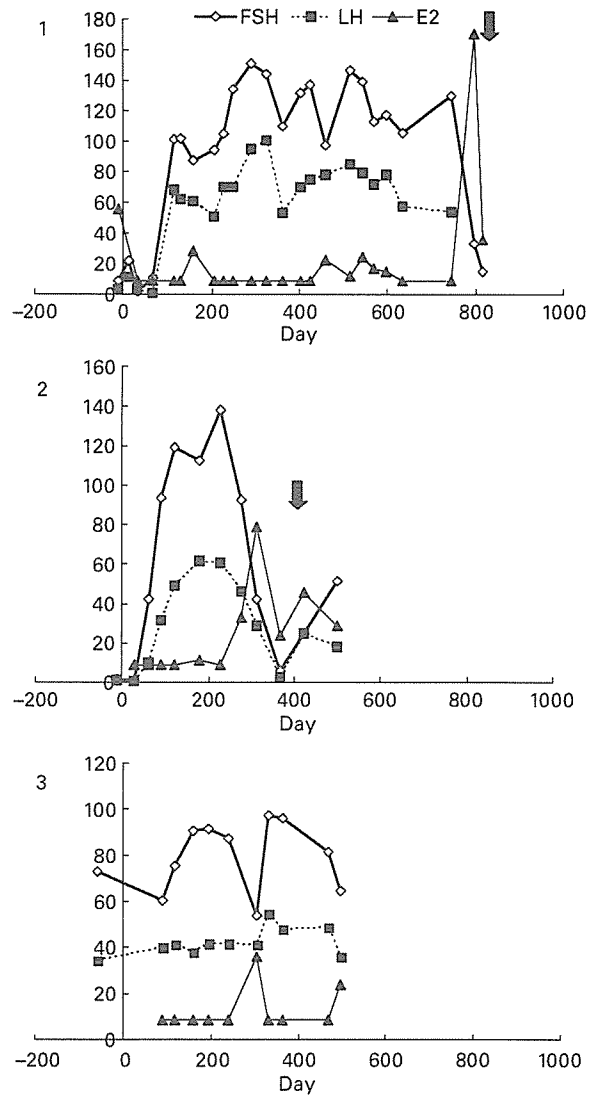

Figure 5 Ovarian function after transplantation. Arrows indicate the day of menstruation recovery.

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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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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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.

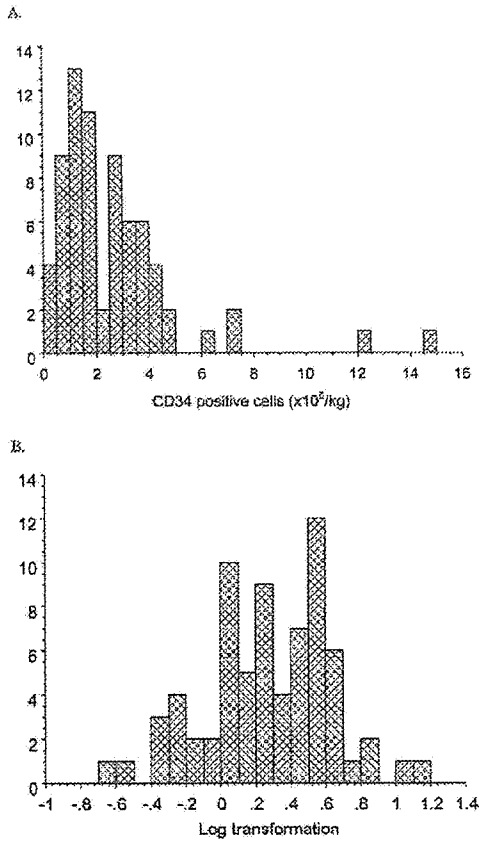

Figure 1. Number of CD34$^+$ cells per donor body weight (kg) collected on day 4 of G-CSF administration before (A) and after (B) logarithmic transformation.

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.

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).

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

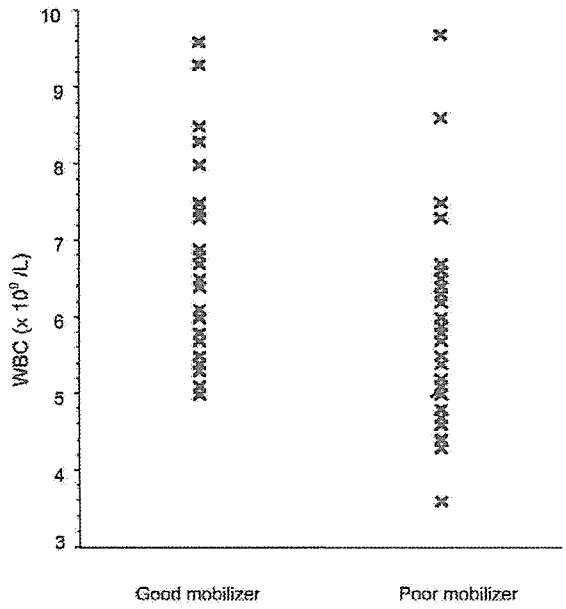

Figure 2. Peripheral blood WBC count before mobilization grouped according to outcome of mobilization. Poor mobilizers were defined as those with collection of less than 2.0×10^6 $CD34^+$ cells per donor body weight (kg) on day 4 of filgrastim administration.

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.

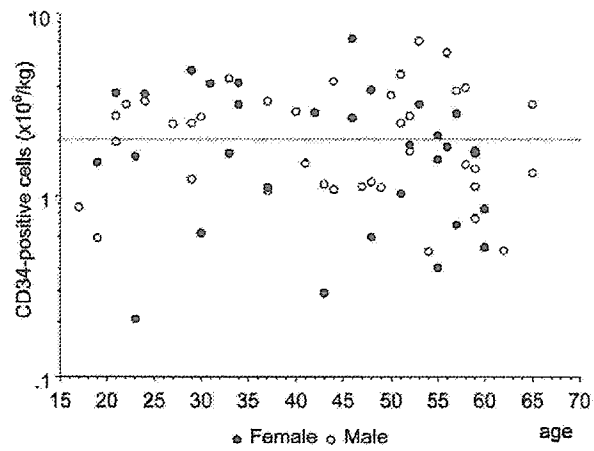

Figure 3. Correlation between age and number of $CD34^+$ cells per donor body weight (kg) collected on day 4 of G-CSF administration.

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