研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Oshima K, Kanda Y, Yamashita T, Takahashi S, Mori T, Nakaseko C, Fujimaki K, Yokota A, Fujisawa S, Matsushima T, Fujita H, Sakura T, Okamoto S, Maruta A, Sakamaki H.	Central nervous system relapse of leukemia after allogeneic hematopoietic stem cell transplantation.	Biol Blood Marrow Transplant.	14	1100-7	2008
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Ⅳ. 研究成果の刊行物・別刷

CLINICAL RESEARCH

Central Nervous System Relapse of Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation

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Little information is available regarding central nervous system (CNS) relapse of adult leukemia after allogeneic hematopoietic stem cell transplantation (HSCT). Therefore, we reviewed the data of 1226 patients with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML) who received first allogeneic HSCT between 1994 and 2004, using the database of the Kanto Study Group for Cell Therapy (KSGCT), and analyzed the incidence, risk factors, and outcome of patients with CNS relapse. Twenty-nine patients developed CNS relapse at a median of 296 (9-1677) days after HSCT with a cumulative incidence of 2.3%. Independent significant factors associated with CNS relapse included ALL as the underlying diagnosis (relative risk [RR] = 9.55, 95% confidence interval [CI] = 1.26-72.2, P = .029), nonremission at HSCT (RR = 2.30, 95% CI = 1.03-5.15, P = .042), the history of CNS invasion before HSCT (RR = 5.62, 95% CI = 2.62-12.0, $P = 9.2 \times 10^{-6}$), and the prophylactic intrathecal chemotherapy after HSCT (RR = 2.57, 95% CI = 1.21-5.46, P = .014). The 3-year overall survival (OS) after CNS relapse was 18%. In 7 of 29 patients with CNS relapse, leukemia was observed only in CNS. Three of 7 patients were alive without systemic relapse, resulting in 3-year survival after CNS relapse of 46%. Although the outcome of patients with CNS relapse was generally poor, long-term disease-free survival could be achieved in some patients.

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KEY WORDS: Leukemia, Central nervous system, Relapse, Allogeneic hematopoietic stem cell transplantation

INTRODUCTION

Relapse of the original disease remains 1 of the most important causes of failure after allogeneic hematopoietic stem cell transplantation (HSCT) for leukemia. Although majority of the patients develop systemic relapse, extramedullary relapse has been also observed after HSCT. The incidence of central nervous system (CNS) relapse after allogeneic HSCT ranged from 2.9% to 11% [1-3]. Risk factors for CNS relapse identified in previous studies included CNS involvement before HSCT [2] and nonremission at HSCT [1]. Prophylactic intrathecal administration of methotrexate (MTX) was shown to decrease the incidence of CNS relapse of acute lymphoblastic leukemia (ALL) in the Seattle study [1], whereas the other 2

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studies failed to find the benefit of prophylactic intrathecal administration of MTX on CNS relapse in patients with acute leukemia [2,3]. There has been no generalized consensus on intrathecal administration of MTX, and in fact, a survey of the European Group for Blood and Marrow Transplantation (EBMT) had reported that the practice varied widely among centers [4].

We examined the incidence, risk factors, and outcome of CNS relapse after allogeneic HSCT in adult patients with acute myelogenous leukemia (AML), ALL, and chronic myelogenous leukemia (CML), and also evaluated the prophylactic effect of intrathecal administration of MTX on CNS relapse.

MATERIALS AND METHODS

Study Population

The study population consisted of 1226 patients, who underwent allogeneic HSCT for AML, ALL, and CML for the first time between January 1994 and December 2004 at 10 hospitals participating in the Kanto Study Group for Cell Therapy (KSGCT).

Transplantation Procedure

Of the 1226 patients, the sources of stem cell was bone marrow (BM) in 903, peripheral blood stem cells (PBSC) in 178, BM plus PBSC in 10, and cord blood (CB) in 134. Conventional myeloablative conditioning regimens such as total body irradiation (TBI) and cyclophosphamide (Cy), busulfan (Bu), and Cy, and their modified regimens were performed in 1168 patients. Among them, TBI of at least 10 Gy was performed in 815 patients. Reduced-intensity conditioning (RIC) regimens were conducted in 53 patients. Prophylaxis of graft-versus-host disease (GVHD) was attempted with calcineurin inhibitors (cyclosporine [CsA] or tacrolimus) with or without short-term MTX in the majority of patients.

Definition of CNS Relapse

CNS relapse was diagnosed as the presence of leukemic cells in the cerebrospinal fluid (CSF). Isolated CNS relapse was defined as CNS relapse without any other sites of relapse of leukemia.

Statistical Considerations

Overall survival (OS) was calculated using the Kaplan-Meier method. Cumulative incidence of CNS relapse was calculated using Gray's method, considering death without CNS relapse as a competing risk [5]. Cumulative incidence of isolated CNS relapse was calculated using Gray's method, treating systemic relapse and death without relapse as a competing risk [5]. The protective effect of chronic GVHD (cGVHD) on CNS relapse was evaluated among patients who developed bone marrow relapse within 100 days after HSCT. Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. Finally, P values of $<.05$ were considered statistically significant.

RESULTS

Characteristics of the Patients

Characteristics of patients included in the study were listed in Table 1. The median age was 36 years, ranging from 15 to 69 years. The underlying diseases were AML (n = 533), ALL (n = 352), and CML (n = 341). Eighty-one patients had the history of CNS involvement before HSCT. Eight hundred and nine patients were in complete remission of acute leukemia or in chronic phase of CML at HSCT, and the remaining patients had active disease. In the following analyses, CML in the chronic phase was included in leukemia in complete remission.

CNS Relapse

Twenty-nine patients developed CNS relapse at a median of 296 days (9-1677 days) after HSCT, giving the cumulative incidence of 2.3% (Figure 1). The median age was 31 years (range: 17-47). The underlying disease was ALL in 18, AML in 9, and CML in 2. Sixteen patients had CNS involvement before HSCT and

Table 1. Characteristics of Patients

Median age (range) at transplantation	36 (15-69)
Sex	
Male	762
Female	464
Underlying disease	
AML	533
ALL	352
CML	341
Disease status	
CR	809
non-CR	416
History of CNS disease	
Yes	81
No	802
Type of conditioning	
Conventional	1168
Reduced intensity	53
TBI ≥ 10 Gy in conditioning	
Yes	815
No	404
Donor type	
Related	478
Unrelated	548
Stem cell source	
BM	902
PBSC	178
BM + PBSC	10
CB	134

CB indicates cord blood.

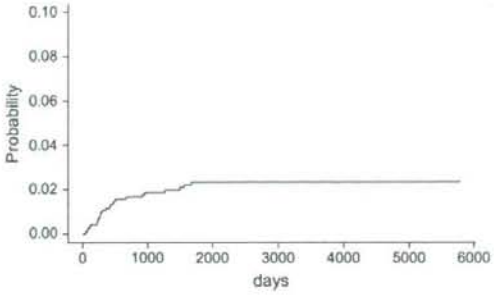

Figure 1. Cumulative incidence of CNS relapse treating death without CNS relapse as competing risk.

6 of them had active CNS disease at HSCT. OS after CNS relapse was 42% at 1 year and 18% at 3 years (Figure 2A). OS of the whole patient cohort, patients with CNS relapse, and those without CNS relapse was 59.8%, 33.2%, and 60.6%, respectively, at 3 years after transplantation.

Pretransplant factors that affected the incidence of CNS relapse after HSCT with at least borderline significance were ALL as the underlying disease, active disease at HSCT, a history of CNS leukemia, the use of TBI regimens, HSCT from an unrelated donor, and the use of prophylactic intrathecal chemotherapy after HSCT (Table 2). Among them, multivariate analysis showed that ALL as the underlying disease, active disease at HSCT, the history of CNS involvement, and the use of intrathecal chemotherapy after HSCT were independently significant (Table 2 and Figure 3). The cumulative incidences of CNS relapse in patients with and without a history of CNS involvement before HSCT were 21.3% and 1.3%, respectively (Figure 3A). Patients with ALL were at higher risk for CNS relapse even in patients in remission at HSCT without a history of CNS involvement before HSCT (ALL 2.7%, AML 0.8%, and CML 0.4%, $P = .088$, Figure 4A). Twenty-three patients who had active leukemia at HSCT had persistent disease after HSCT. Among these, only 2 patients developed CNS relapse after HSCT. However, median survival of this cohort was only 90 days after HSCT producing a 1-year survival of 14%, and thus, majority of the patients died very early, before developing CNS relapse.

Effect of Intrathecal Chemotherapy on the Incidence of CNS Relapse

The practice of intrathecal chemotherapy in allogeneic HSCT recipients varied among the 10 institutions of the KSGCT. Half of them never used prophylactic intrathecal chemotherapy before and after HSCT. The remaining half administered intrathecal prophylaxis routinely before HSCT, of which 2 institutions added intrathecal chemotherapy after

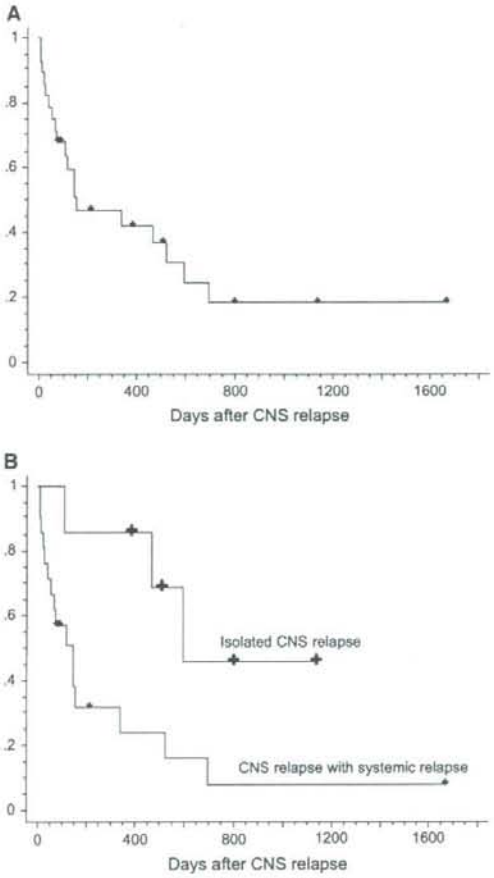

Figure 2. OS after CNS relapse (A) and that grouped according to isolated CNS relapse or CNS relapse associated with systemic relapse (B).

HSCT for high-risk patients such as those with ALL or the history of CNS involvement. In this cohort, intrathecal prophylaxis before HSCT was conducted in 701 of 887 patients and intrathecal chemotherapy after HSCT was done in 141 of 807 patients whose information about intrathecal chemotherapy was available. Antineoplastic agents used for intrathecal chemotherapy mainly consisted of MTX. The median numbers of intrathecal chemotherapy before and after HSCT were 1 (range: 1-4) and 2 (range: 1-4), respectively.

We failed to find a significant prophylactic effect of intrathecal chemotherapy for CNS relapse. The relative risk for CNS relapse was 1.52 (95% CI 0.61-3.79, $P = .37$) for intrathecal chemotherapy before HSCT and 3.92 (95% CI 1.80-8.51, $P = .00057$) for intrathecal chemotherapy after HSCT (Table 2). This adverse influence of intrathecal chemotherapy after HSCT was significant even after adjusted for the

Table 2. Impact of Pretransplant Factors on the Incidence of CNS Relapse after Transplantation

Factor		Univariate RR (95% CI)	P value	Multivariate RR (95% CI)	P value
Age		1.00 (1.00-1.00)	.15		
Sex		1.01 (0.65-1.59)	.95		
Disease	CML	1.00		1.00	
	AML	5.58 (0.70-44.5)	.10	3.60 (0.46-28.4)	.22
	ALL	17.7 (2.36-132.8)	.0052	9.55 (1.26-72.2)	.029
CR/non-CR		2.33 (1.08-5.04)	.031	2.30 (1.03-5.15)	.042
History of CNS disease		17.9 (8.30-38.6)	2.0×10^{-13}	5.62 (2.62-12.0)	9.2×10^{-6}
TBI		2.91 (1.00-8.44)	.050		
Conventional/reduced intensity		0.99 (0.47-2.07)	.97		
Related/unrelated		1.85 (1.06-3.23)	.030		
Source	BM	1.00			
	PBSC	0.24 (0.03-1.77)	.16		
	CB	0.70 (0.17-2.96)	.63		
Sex mismatch		1.06 (0.42-2.66)	.90		
HLA mismatch		0.46 (0.06-3.46)	.45		
Prophylactic IT before HSCT		1.52 (0.61-3.79)	.37		
Prophylactic IT after HSCT		3.92 (1.80-8.51)	.00057	2.57 (1.21-5.46)	.014

IT indicates intrathecal chemotherapy; CNS, central nervous system; RR, relative risk.

underlying disease, disease status at HSCT, and the history of CNS involvement before HSCT (relative risk 2.57, 95% CI 1.21-5.46, $P = .014$). Among patients without a history of CNS involvement before HSCT who were in remission at HSCT, the incidences of CNS relapse after HSCT were 3.6% and 1.6% who received and did not receive intrathecal chemotherapy after HSCT, respectively ($P = .057$, Figure 4B). In patients with a history of CNS involvement before HSCT, the incidences of CNS relapse after HSCT were 37.4% and 11.6%, respectively, who received and did not receive intrathecal chemotherapy after HSCT ($P = .018$; Figure 4C). When we limited the analysis in patients with ALL, the incidences of CNS relapse after HSCT were 6.2% and 3.7% who received and did not receive intrathecal chemotherapy after HSCT ($P = .17$), respectively, in patients without a history of CNS involvement before HSCT who were in remission at HSCT and they were 55.6% and 15.5%, respectively, in patients with a history of CNS involvement before HSCT ($P = .0081$).

Nine patients developed leukoencephalopathy with a median onset of 288 days after HSCT. The incidence of leukoencephalopathy was significantly higher in patients who underwent intrathecal chemotherapy after HSCT (3.5% versus 0.5%, $P = .0076$).

Isolated CNS Relapse

Seven patients developed isolated CNS relapse at a median of 671 days (125-1677 days) after HSCT, presenting the cumulative incidence of 0.70%. Characteristics of these 7 patients were listed in Table 3. All received bone marrow as stem cell source. Prognostic factors associated with isolated CNS relapse with at least borderline significance were age, active disease at HSCT, CNS involvement before HSCT, stem cell source, the use of intrathecal chemotherapy after HSCT, and the absence of HLA mismatch. Among these, independent significant factors for isolated CNS relapse included the history of CNS involvement before HSCT, the use of PBSC or CB as stem cell source, and the absence of HLA mismatch (Table 4). The treatment of isolated CNS relapse consisted of intrathecal chemotherapy and/or cranial irradiation and CNS disease was successfully controlled in 5 of the 7 patients. Four patients developed bone marrow relapse within 1 year. However, the remaining 3 patients were alive without systemic relapse at 518, 807, and 1149 days after CNS relapse and 1283, 1478, and 2195 days after HSCT, respectively. Survival after CNS relapse was significantly better in patients who developed isolated CNS relapse than those who developed CNS relapse with systemic relapse (46% versus 8% at 3 years, $P = .023$, Figure 2B).

Effect of cGVHD on CNS Relapse

Among the 378 patients who experienced bone marrow relapse within 100 days after HSCT but were free from CNS relapse at day 100, 21 (6.1%) showed CNS relapse later on. The incidence of CNS relapse after bone marrow relapse was 7.1% in patients with cGVHD and 2.0% in those without cGVHD ($P = .14$).

Analysis Excluding CML Patients

We repeated these analyses excluding patients with CML, because the incidence of CNS relapse was extremely low, as shown in Figure 3C. The cumulative incidence of CNS relapse was 3.2%. Independently significant pretransplant factors for CNS relapse

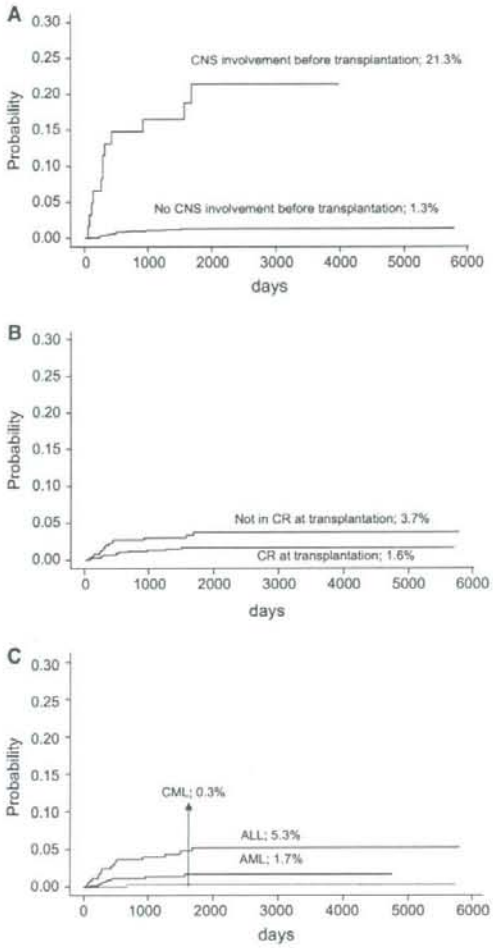

Figure 3. Cumulative incidence of CNS relapse grouped according to the history of CNS involvement before transplantation (A), disease status at transplantation (B), and underlying disease (C).

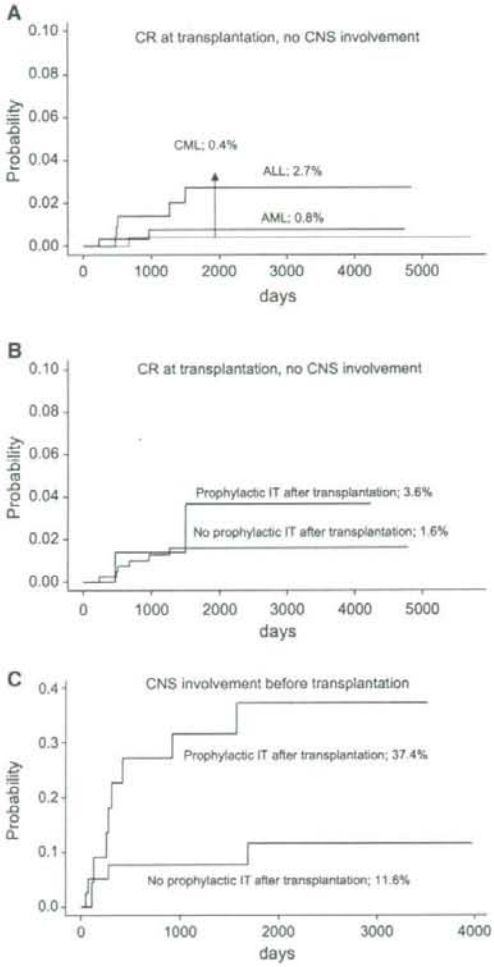

Figure 4. Cumulative incidence of CNS relapse in patients in remission at transplantation without a history of CNS involvement before transplantation grouped according to the underlying disease (A) and the use of prophylactic intrathecal chemotherapy (IT) after transplantation (B). Cumulative incidence of CNS relapse in patient with CNS involvement before transplantation grouped according to the use of prophylactic IT after transplantation (C).

were the same as the analyses including CML patients; ALL compared to AML as the underlying disease (RR 2.68, 95% CI 1.18-6.11, $P = .019$), active disease at HSCT (RR 2.49, 95% CI 1.08-5.73, $P = .032$), the history of CNS involvement (RR 5.64, 95% CI 2.60-12.3, $P = .000012$), and the use of intrathecal chemotherapy after HSCT (RR 2.69, 95% CI 1.25-5.81, $P = .012$). The cumulative incidence of isolated CNS relapse was 0.9%. Independently significant pretransplant factors for CNS relapse included ALL compared to AML as the underlying disease, the history of CNS involvement, the use of PBSC as stem cell source, the absence of HLA mismatch, and the use of intrathecal chemotherapy after HSCT.

DISCUSSION

The cumulative incidences of CNS relapse and isolated CNS relapse were 2.3% and 0.70% in this cohort, respectively, which were almost comparable with those in previous studies (Table 5) [1-3]. The history of CNS leukemia before HSCT was identified as the strongest predictive factor for CNS relapse after HSCT in our study as previously reported [1,2].

We could not show a beneficial effect of prophylactic intrathecal chemotherapy on the incidence of

Table 3. Characteristics of Patients Who Developed Isolated CNS Relapse after Transplantation

Patient No.	1	2	3	4	5	6	7
Age	23	31	24	35	26	41	18
Sex	M	M	M	M	F	M	M
Disease	CML	CML	ALL	AML	ALL	ALL	ALL
Disease status	CP2	BC	RL2	RL1	RL2	CR1	RL2
History of CNS disease	Yes	Yes	Yes	Yes	Yes	No	No
Stem cell source	BM	BM	BM	BM	BM	BM	BM
Donor type	R	R	U	R	R	R	U
HLA mismatch	No	Yes	No	No	No	No	Yes
Conditioning regimem	Bu+Cy	CA+Cy+TBI	ETP+Cy+TBI	CA+Cy+TBI	ETP+Cy+TBI	Cy+TBI	CA+Cy+TBI
Days to an isolated CNS relapse	671	134	125	1565	276	1265	1677
CNS treatment	IT+RT	IT+RT	IT	IT+DLI	RT	IT+RT	IT
Systemic relapse	No	No	Yes	Yes	Yes	Yes	No
Days from HSCT to systemic relapse	164	1680	444	1572			
Day from CNS relapse to systemic relapse	39	115	168	307			
Outcome	Alive	Alive	Dead	Dead	Dead	Alive	Alive
Follow-up duration (days)	1478	1283	236	2031	870	1661	2195

IT indicates intrathecal chemotherapy; RT, radiation; DLI, donor lymphocyte infusion; BU, busulfan; CY, cyclophosphamide; CA, cytarabine; ETP, etoposide; CNS, central nervous system; HSCT, hematopoietic stem cell transplantation.

CNS relapse after HSCT. The incidence of CNS relapse was rather higher in patients who received intrathecal chemotherapy after HSCT. This was probably biased by the fact that significantly higher proportion of patients received intrathecal chemotherapy after HSCT among patients with CNS involvement before HSCT than those without CNS leukemia (47.4% versys 13.4%, $P < .0001$). However, intrathecal chemotherapy after HSCT significantly adversely affected the incidence of CNS relapse even after adjusted for the underlying disease, disease status at HSCT, and the history of CNS involvement before HSCT. Also, a benefit of intrathecal chemotherapy after HSCT was not shown in patients with ALL, in contrast with the previous reports [1,6]. This discrepancy might have resulted from the difference in the intensity of the intrathecal chemotherapy. Intrathecal chemotherapies were administered 6 times after HSCT in the Seattle group, whereas the medium number of intrathecal chemotherapy in the current study was only 2 (range: 1-4). Therefore, the intensity of intrathecal chemotherapy might be important to sufficiently prevent CNS relapse after HSCT. However, they observed the development of leukoencephalopathy in 7 of the 415 patients and we also observed leukoencephalopathy significantly more frequently in patients who received intrathecal chemotherapy after HSCT than those who did not. Therefore, such an intensive intrathecal chemotherapy should be avoided for patients at low risk for CNS relapse. We had a concern that the use of intrathecal chemotherapy after HSCT might delay immune recovery and thereby

Table 4. Impact of Pretransplant Factors on the Incidence of Isolated CNS Relapse after Transplantation

Factor		Univariate RR (95% CI)	P-Value	Multivariate RR (95% CI)	P-Value
Age		0.99 (0.98-1.00)	.055		
Sex		1.05 (0.47-2.34)	.90		
Disease	CML	1.00			
	AML	0.73 (0.04-11.9)	.82		
	ALL	5.31 (0.61-45.9)	.13		
CR/non-CR		4.98 (0.97-25.7)	.055		
History of CNS disease		48.3 (9.37-249.4)	3.6×10^{-6}	48.1 (9.40-245.9)	3.3×10^{-6}
TBI		3.21 (0.38-26.8)	.28		
Conventional/reduced intensity		1.08 (0.26-4.49)	.92		
Related/unrelated		1.45 (0.65-3.24)	.37		
Source	BM	1.00		1.00	
	PBSC	N.A.	<.0001	N.A.	<.0001
	CB	N.A.	<.0001	N.A.	<.0001
Sex mismatch		1.58 (0.26-9.41)	.62		
HLA mismatch		N.A.	<.0001	N.A.	<.0001
Prophylactic IT before HSCT		1.09 (0.21-5.61) 0.92			
Prophylactic IT after HSCT		7.11 (1.62-31.2) 0.0094			

N.A. indicates not assessable because no events were observed in the group; IT, intrathecal chemotherapy; RR, relative risk.

Table 5. Cumulative Incidence of CNS Relapse after HSCT in Prior Studies and Our Study

	n	Underlying Disease (AML/ALL/CML)	History of CNS Leukemia (%)	CR at Transplant (%)	Allogeneic Transplant (%)	Incidence of CNS Relapse (%)	Reference
1	415	217/198/0	23.4	47.7	100	2 in AML, 13 in ALL	1
2	92	0/92/0	22.8	100	71.7	11	2
3	487	366/121*/0	3.5	100	67.6	2.9	3
4	1226	533/352/341	9.2	65.8	100	2.3	Present report

*Including 5 patients with acute unclassified leukemia.

increase the risk of systemic relapse, but the incidence of systemic relapse was not significantly different between those who received intrathecal chemotherapy and those who did not (relative risk 1.11, 95% CI 0.79-1.55, $P = .56$). The use of total body irradiation (TBI) in the conditioning regimen has been considered to prevent CNS relapse, because irradiation is effective for so called sanctuary sites of chemotherapy. However, the incidence of CNS relapse was also rather higher in patients who received the TBI regimen. This may be again because of the fact that significantly higher proportion of patients received the TBI regimen among patients with CNS involvement before HSCT than those without CNS leukemia (81.5% versus 57.9%, $P < .0001$).

As for stem cell source, isolated CNS relapse was observed exclusively after BMT. A possible explanation for this may be the year effect, because allogeneic PBSCT and CBT started after 2000 in Japan. However, the year of HSCT of patients who developed isolated CNS relapse evenly ranged between 1997 and 2002. Another possible explanation is the presence of graft-versus-CNS relapse effect enhanced by increased incidence of cGVHD after allogeneic PBSCT and the presence of HLA-mismatch in CBT. The significantly higher incidence of CNS relapse after autologous HSCT than that after allogeneic HSCT suggested the existence of such an immunologicprotection against CNS relapse [2]. Isolated extramedullary relapse was also reported to be observed earlier in autologous HSCT than in allogeneic HSCT [7]. Furthermore, successful treatment of CNS relapse with reduced-intensity transplantation may suggest the presence of graft-versus-leukemia CNS leukemia effect [8], although the other reports doubted such effect against for CNS lesions [9-12]. The observed tendency toward a lower CNS relapse incidence after bone marrow relapse in patients with cGVHD than those without cGVHD in the current study might support this speculation, although we have no immunologic evidence.

The prognosis of patients who developed relapse after allogeneic HSCT has been reported to be extremely poor [13,14]. Also, survival after isolated CNS relapse was reported to be no better than that after bone marrow relapse in pediatric patients with AML and adult patients with ALL [15,16]. However, in the current study, 3 of the 7 patients who developed isolated CNS relapse were alive for more than a year without leukemia, resulting in the significantly better survival than those who developed CNS relapse after or simultaneously with systemic relapse. We could not identify the reason for this discrepancy, but the age and underlying disease of the study population differed between our study and the previous report. We consider that an intensive treatment against CNS leukemia is warranted for adult patients with isolated CNS relapse.

In conclusion, we confirmed that ALL as the underlying disease, active disease at HSCT, and the history of CNS involvement before HSCT were significant predictors for CNS relapse after HSCT. We failed to show a significant prophylactic effect of intrathecal chemotherapy to prevent CNS relapse and such a prophylactic treatment should be avoided for patients at low risk for CNS relapse. The prognosis for isolated CNS relapse was surprisingly good.

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Decreased incidence of acute graft-versus-host disease by continuous infusion of cyclosporine with a higher target blood level

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Cyclosporine A (CsA) is the mainstay of pharmacologic prevention of acute graft-versus-host disease (GVHD). We previously reported that continuous infusion of CsA with a target blood level between 250 and 400 ng/ml significantly increased the incidence of acute GVHD compared to twice-daily infusion with a target trough level between 150 and 300 ng/ml. Thus, we raised the target level of CsA continuous infusion to 450–550 ng/ml. We treated 33 patients with the higher target level (CsA500) and compared the efficacy and toxicity with those in the 33 historical control patients (CsA300 group). Other transplantation procedures were not changed. The patients' characteristics were equivalent. The average CsA concentration was adjusted around 500 ng/ml and the actual daily dose was maintained at the initial dose (CsA 3mg/kg/day). Toxicities were equivalently observed among the two groups. The incidence of grades II–IV acute GVHD was significantly lower in the CsA500 group (27 vs. 52%, $P = 0.033$). The target level of CsA was identified as an independent significant risk factor for grades II–IV acute GVHD ($P = 0.039$), adjusted for the presence of HLA mismatch. The incidence of chronic GVHD was also decreased in the CsA500 group (47 vs. 73%, $P = 0.016$). We conclude that the toxicity of the continuous CsA infusion with a target level of 450–550 ng/ml is acceptable and the efficacy to prevent acute GVHD is significant. A larger comparative study is warranted to confirm these findings. Am. J. Hematol. 83:226–232, 2008. © 2007 Wiley-Liss, Inc.

Introduction

Cyclosporine A (CsA) is one of the most commonly used immunosuppressive agents for the prevention of acute graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). However, the dose, target blood level, and schedule of infusion vary among protocols and have not been optimized [1]. On the other hand, the importance of blood CsA concentration as well as administered dose has been shown in several reports [2–5]. We previously compared continuous infusion of CsA with a target blood level between 250 and 400 ng/ml and twice-daily infusion targeted to a trough level between 150 and 300 ng/ml in the early period after transplantation in a retrospective study [6]. The incidence of grades II–IV acute GVHD was significantly higher in patients who received the continuous CsA infusion, adjusted for the other significant factors. The actual daily dose of CsA in the continuous infusion group was decreased from the starting dose of 3–1.9 mg/kg/day on average at 4 weeks after transplantation, which might have adversely affected the incidence of acute GVHD. However, the incidences of renal dysfunction and relapse were significantly lower in these patients. The lower incidence of relapse in the continuous infusion group resulted in better disease-free survival in patients with high-risk diseases (43 vs. 16% at 2 years, $P = 0.039$), but not in standard-risk patients (72 vs. 80%, $P = 0.45$). We thus considered that the target CsA level of 250–400 ng/ml in the continuous infusion group was appropriate in high-risk patients, but too low in standard-risk patients. Therefore, we raised the target level of CsA to 450–550 ng/ml when we continuously infuse CsA in standard-risk patients [7]. In this report, we evaluated the safety and efficacy of the continuous infusion of CsA with this high target blood concentration at 500 ng/ml.

Results

Patient characteristics

We performed allogeneic HSCT for 33 standard-risk patients with the higher target CsA level at 450–550 ng/ml (CsA500 group). The historical control group treated with the original target CsA level at 250–400 ng/ml (CsA300 group) also included 33 patients [6]. The characteristics of the patients were equivalent between the two groups, except for the underlying disease (Table I). The number of patients with chronic myelogenous leukemia (CML) was only 2 in the CsA500 group, including one with chronic neutrophilic leukemia in uncontrollable leukocytosis, due to the introduction of imatinib in the treatment of such patients.

Blood concentration and actual daily dose of CsA

The dose of CsA was adjusted to maintain the blood CsA concentration between 450 and 550 ng/ml in the CsA500 group. All patients required repeated dose adjustments of CsA to maintain the targeted blood level. This adjustment was successful and the mean CsA concentration was 488 ±

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TABLE I. Characteristics of the Patients

	CsA500 group (n = 33)	CsA300 group (n = 33)	P-value
Sex			
Male	20	26	0.18
Female	13	7	
Age			
<40	16	17	>0.99
≥40	17	16	
Underlying disease			
AL	24	13	0.017
CML	2	12	
MDS	2	1	
NHL	3	6	
Others	2	1	
Donor			
Related	12	16	0.46
Unrelated	21	17	
HLA			
Match	28	25	0.54
Mismatch	5	8	
Stem cell source			
BM	25	26	>0.99
PB	8	7	
Regimen			
Non-TBI	4	9	0.21
TBI	29	24	
MTX dose			
<31mg/m^2	16	11	0.32
≥31mg/m^2	17	22	

BM, bone marrow; PB, peripheral blood; TBI, total body irradiation; AL, acute leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma.

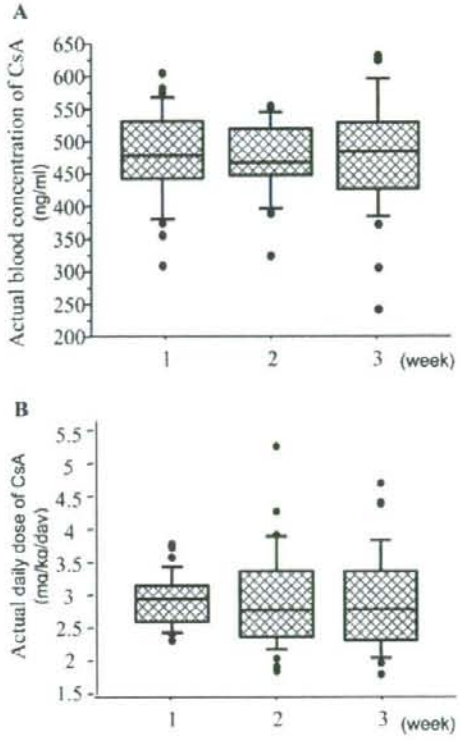

Figure 1. Actual blood concentration (A) and daily dose (B) of cyclosporine. The mean CsA concentration was 488 ± 89, 475 ± 41, and 482 ± 69 ng/ml and the actual dose was 2.9 ± 0.4, 2.8 ± 0.8, and 2.7 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after HSCT, respectively.

89, 475 ± 41, and 482 ± 69 ng/ml at the 1st, 2nd, and 3rd week after HSCT, respectively (Fig. 1A). The actual dose was 2.9 ± 0.4, 2.8 ± 0.8, and 2.7 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after HSCT, respectively (Fig. 1B). The median duration of intravenous cyclosporine was 41 days (range 16–74 days) after transplantation.

Toxicity

The incidence of renal dysfunction defined as elevation of the serum creatinine level above ×1.5 and ×2.0 the baseline value was equivalent between the CsA500 group and the CsA300 group (Table II, 24 vs. 24%, P = 0.96 and 15 vs. 13%, P = 0.71, respectively). Liver dysfunction defined as elevation of the total bilirubin level above 2 mg/dl was also similar (30 vs. 24%, P = 0.78). Thrombotic microangiopathy was not observed in any patients. No central nerves system toxicities were observed. In the CsA500 group, we decreased the target level of CsA to 300 ng/ml due to hyperbilirubinemia 9 days after HSCT in one patient and substituted prednisolone for CsA in another patient due to hyperbilirubinemia and renal dysfunction at day 21 after HSCT. The latter patient had already had liver cirrhosis classified to Child-Pugh A due to hepatitis C virus infection before HSCT.

TABLE II. Incidences of Adverse Events Due to Cyclosporine

	(−)	(+)	P-value
Incidence of serum creatinine > 1.5 × baseline value			
CsA500	25	8 (24%)	>0.99
CsA300	25	8 (24%)	
Incidence of serum creatinine > 2.0 × baseline value			
CsA500	28	5 (15%)	0.71
CsA300	30	3 (13%)	
Incidence of bilirubin > 2.0 mg/dl			
CsA500	23	10 (30%)	0.78
CsA300	25	8 (24%)	
Incidence of TMA			
CsA500	33	0 (0%)	>0.99
CsA300	33	0 (0%)	

TMA: thromobtic microangiopathy.

Incidences of acute and chronic GVHD

We performed a univariate analysis to evaluate the impact of potential confounding factors on the incidence of grades II–IV acute GVHD and identified two significant factors; the presence of HLA-mismatch including allele-mismatch and the target level of CsA (Table IIIA). As shown in Fig. 2A, the incidence of grades II–IV acute GVHD in the CsA300 group was significantly higher than that in the CsA500 group (52 vs. 27%, P = 0.033). Corticosteroids therapy for acute GVHD was more frequently required in the CsA300 group (39 vs. 15%, P = 0.051). The percentage of patients who received corticosteroids to treat GVHD was lower than the incidence of grades II–IV acute GVHD, because we did not use systemic corticosteroids for grades II acute GVHD with skin involvement only. The difference in the incidence of

TABLE III. Factors Associated the Incidences of Grades II–IV Acute GVHD and Nonrelapse Mortality

A. Univariate analyses

Factor	Acute GVHD	*P*-value	Nonrelapse mortality	*P*-value
Sex				
Male	20 (44%)	0.31	12 (30%)	0.020
Female	6 (30%)		0 (0%)	
Age				
<40 years	15 (46%)	0.30	4 (14%)	0.21
≥40 years	11 (33%)		8 (30%)	
Underlying disease				
CML	7 (50%)	0.25	2 (14%)	0.49
Non-CML	19 (37%)		10 (25%)	
Donor				
Related	11 (39%)	0.97	8 (36%)	0.052
Unrelated	15 (40%)		4 (13%)	
HLA				
Match	17 (32%)	0.0037	10 (23%)	0.78
Mismatch	9 (69%)		2 (18%)	
Stem cell source				
BM	19 (37%)	0.46	9 (21%)	0.68
PBSC	7 (47%)		3 (24%)	
Regimen				
Non-TBI	4 (31%)	0.56	5 (49%)	0.035
TBI	22 (42%)		7 (15%)	
MTX dose				
<31mg/m^2	12 (44%)	0.32	7 (19%)	0.87
≥31mg/m^2	14 (36%)		5 (24%)	
Target levels of CsA				
CsA500	9 (27%)	0.033	2 (8%)	0.051
CsA300	17 (52%)		10 (27%)	

B. Multivariate analyses

Factor	RR of acute GVHD	*P*-value	RR of nonrelapse mortality	*P*-value
Target levels of CsA				
CsA300	1.00	0.039	1.00	0.064
CsA500	0.43 (0.19–0.96)		0.24 (0.053–1.09)	
HLA				
Match	1.00	0.0062		
Mismatch	3.14 (1.39–7.14)			

grades II–IV acute GVHD between the two groups was more prominent in unrelated HSCT (Fig. 2B, 44 vs. 33% in related HSCT and 59 vs. 24% in unrelated HSCT).

Next, we performed a multivariate analysis to identify independent risk factors for the development of Grades II–IV acute GVHD. Two factors were independently significant with a relative risk (RR) of 3.14 (95% confidence interval [CI] 1.39–7.14, $P = 0.0062$) for the presence of HLA-mismatch and RR of 0.43 (95% CI 0.19–0.96, $P = 0.039$) for the CsA500 group, respectively (Table IIIB). The cumulative incidence of Grades III, IV acute GVHD was only 11%. The target level of cyclosporine (CsA500 vs. CsA300: 3 vs. 18%, $P = 0.045$) was identified as the only significant risk factor for the development of Grades III, IV acute GVHD.

The number of patients who developed limited and extensive chronic GVHD was 5 and 18, respectively, in the CsA300 group and 4 and 11, respectively, in the CsA500 group. The incidence of chronic GVHD was also significantly decreased in the CsA500 group (Table IV and Fig. 3, 47 vs. 73%, $P = 0.016$).

Transplantation outcome

The lower incidence of acute GVHD in the CsA500 group translated into the lower incidence nonrelapse mortality (Table III, 8 vs. 27%, $P = 0.051$). On the other hand, the incidence of relapse tended to be higher in the CsA500 group (Table V, 20 vs. 6%, $P = 0.065$), although this difference became smaller when we excluded patients with CML (19 vs. 10%, $P = 0.29$). Finally, there was no significant difference in disease-free survival between the CsA500 group and the CsA300 group (Fig. 4, 72 vs. 63%, $P = 0.68$).

Discussion

We successfully maintained the blood CsA concentration at around 500 ng/ml and the actual dose at around 3 mg/kg/day by twice a week monitoring for the first 3 weeks after transplantation. The preliminary data in these 33 patients suggested the feasibility and efficacy of the continuous infusion of CsA at this higher target level.

Several studies have reported the relationship between the blood concentration of CsA and the efficacy to prevent GVHD after allogeneic HSCT [2–5]. Especially, the area under the concentration–time curve (AUC) has been believed to be the most important pharmacokinetic parameter for the efficacy of calcineurin inhibitors [8,9]. The monitoring of AUC, however, requires frequent blood sampling and is not suitable for daily practice. Therefore, the trough concentration (C_{TL}) has been measured as a surrogate for AUC in twice-daily infusion of CsA, although recent reports

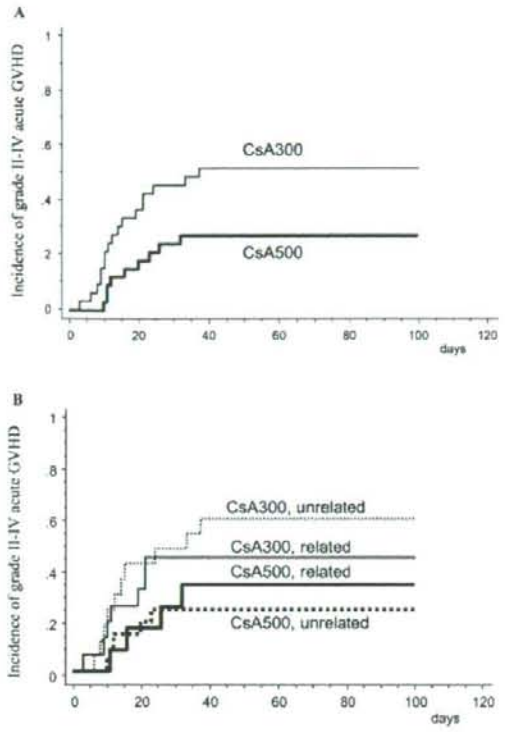

Figure 2. Incidence of Grades II–IV acute GVHD grouped according to the target level of cyclosporine. (A) all patients, (B) stratified by the donor type.

suggested that the measurement of blood concentration at 2–4 hr after infusion may be more appropriate [10]. In continuous infusion, the intradaily variation of the blood concentration of CsA should be minimal and we can evaluate the blood concentration regardless of the timing (steady-state concentration; C_{SS}). However, the relationship between Css in continuous infusion and C_{TL} in twice-daily infusion has not been clarified. Recently, Nakamura et al. reported that the target Css in the continuous infusion of CsA should be 2.55 times the C_{TL} to provide an equal AUC during the twice-daily infusion with a target C_{TL} [11]. Therefore, for example, the target C_{SS} in the continuous infusion of CsA should be 383–638 ng/ml to obtain a similar AUC during the twice-daily infusion with a target C_{TL} at 150–250 ng/ml, that is generally used in daily practice. However, the target blood concentration between 250 and 350 ng/ml is widely used in the continuous infusion of CsA [4]. The expected AUC will be far lower than that during the twice-daily infusion of CsA at the generally used target level. The target C_{SS} in this study at 500 ng/ml (450–550 ng/ml) would be appropriate according to the calculation model. In fact, the actual dose of CsA was maintained at 2.7 and 3.0 mg/kg on average. We had a concern that the incidence of renal dysfunction would be increased, since the relationship between the blood CsA level and drug-induced nephrotoxicity has been shown [12]. The incidence of renal dysfunction, however, was not increased by the dose adjustment and appropriate hydration when CsA levels above the target range were observed.

TABLE IV. Factors Associated the Incidence of Chronic GVHD

A. Univariate analyses

Factor	Chronic GVHD	*P*-value
Sex		
Male	24 (67%)	0.63
Female	10 (56%)	
Age		
<40 years	16 (60%)	0.31
≥40 years	18 (70%)	
Underlying disease		
CML	10 (77%)	0.12
Non-CML	24 (60%)	
Donor		
Related	14 (63%)	0.75
Unrelated	20 (66%)	
HLA		
Match	28 (64%)	0.74
Mismatch	6 (58%)	
Stem cell source		
BM	25 (60%)	0.20
PBSC	9 (81%)	
Regimen		
Non-TBI	27 (64%)	0.75
TBI	7 (65%)	
MTX dose		
<31mg/m²	15 (64%)	0.81
≥31mg/m²	19 (68%)	
Target levels of CsA		
CsA500	11 (47%)	0.016
CsA300	23 (73%)	

B. Multivariate analyses

Factor	RR	*P*-value
Target levels of CsA		
CsA300	1.00	0.014
CsA500	0.44 (0.23–0.85)	

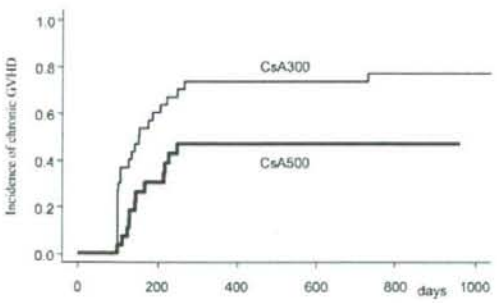

Figure 3. Incidence of chronic GVHD grouped according to the target level of cyclosporine.

Previous randomized control studies that compared continuous infusion of CsA and tacrolimus as GVHD prophylaxis had showed the superiority of tacrolimus to prevent acute GVHD [13–16]. However, these studies employed the lower target level of CsA between 150 and 400 ng/ml. Yanada et al. have also reported that tacrolimus-based regimen was better than cyclosporine-based regimen to prevent GVHD in unrelated bone marrow (BM) transplantation in Japan [17]. However, it was a retrospective analysis

TABLE V. Factors Associated the Incidence of Relapse and Disease-Free Survival

A. Univariate analyses

Factor	Relapse	*P*-value	Disease-free survival	*P*-value
Sex				
Male	5 (12%)	0.90	29 (58%)	0.054
Female	2 (15%)		18 (85%)	
Age				
<40 years	4 (16%)	0.72	25 (71%)	0.35
≥40 years	3 (10%)		22 (60%)	
Underlying disease				
CML	1 (7%)	0.51	11 (79%)	0.34
Non-CML	6 (15%)		36 (60%)	
Donor				
Related	1 (4%)	0.10	19 (60%)	0.53
Unrelated	6 (20%)		28 (67%)	
HLA				
Match	6 (13%)	0.74	37 (63%)	0.64
Mismatch	1 (10%)		10 (72%)	
Stem cell source				
BM	7 (16%)	0.15	35 (63%)	0.55
PBSC	0 (0%)		12 (76%)	
Regimen				
Non-TBI	0 (0%)	0.14	8 (51%)	0.41
TBI	7 (16%)		39 (69%)	
MTX dose				
<31 g/m^2	1 (4%)	0.071	21 (77%)	0.19
≥31 g/m^2	6 (21%)		26 (55%)	
Target levels of CsA				
CsA500	5 (20%)	0.069	26 (72%)	0.68
CsA300	2 (6%)		21 (63%)	

B. Multivariate analyses

Factor	RR of relapse	*P*-value	RR of disease-free survival	*P*-value
Target levels of CsA				
CsA300	1.00	0.065	1.00	0.68
CsA500	4.08 (0.92–18.1)		0.82 (0.32–2.12)	

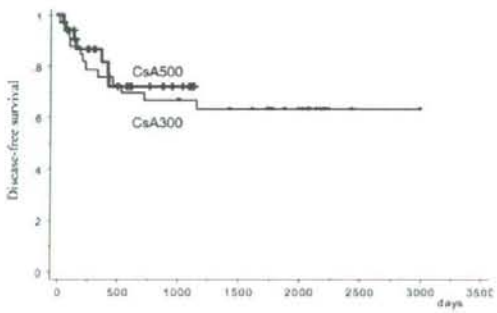

Figure 4. Disease-free survival grouped according to the target level of cyclosporine.

using the database of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and therefore the dose, target blood level, and infusion schedule of both cyclosporine and tacrolimus were various. Especially, the target level of CsA is generally low in the daily practice in Japan. Therefore, the results of these previous studies that compared CsA and tacrolimus as GVHD prophylaxis might have been affected by the target blood concentration [13–17].

The incidence of grades II–IV acute GVHD in the CsA500 group in this study was suppressed to 24% in unrelated HSCT including three HLA allele-mismatched transplants. This incidence was similar to that in the tacrolimus group of patients who underwent HSCT from an alternative donor (30 from an HLA-matched unrelated donor and 4 from the other alternative donor) in a Japanese randomized controlled trial (21%) [13]. Adverse drug reactions were more frequently observed in the tacrolimus group than in the CsA group in this Japanese randomized trial [13], whereas the toxicities in the CsA500 group were equivalent to those in the CsA300 group in the current study. Therefore, the continuous infusion of CsA with a target concentration at 500 ng/ml may provide similar efficacy of GVHD prophylaxis with less frequent toxicities compared to tacrolimus. Wingard et al. have reported that an important relationship between blood concentration of these agents and their efficacy and toxicity using data of a randomized controlled trial [16]. They showed that the efficacy of CsA to prevent GVHD could be improved by elevating the target blood concentration of CsA, whereas the toxicity of tacrolimus could be reduced by lowering the target blood concentration of tacrolimus. Therefore, a randomized controlled trial to compare CsA and tacrolimus with their appropriate target blood concentration is required to draw a definite conclusion.

Another concern about the elevation of the target concentration of CsA was the possible increase in the inci-

dence of relapse [18,19]. We previously showed that the incidence of relapse was significantly lower after the continuous infusion of CsA with the low target CsA concentration at 300 ng/ml compared to twice-daily infusions targeted to 150–300 ng/ml, because the actual dose of CsA was obviously decreased in the continuous infusion group [6]. In this study, the incidence of relapse tended to be higher in the CsA500 group (20 vs. 6%, $P = 0.065$), although there was no significant difference in disease-free survival. A possible explanation of the tendency toward higher relapse rate in the CsA500 group was the impaired graft-versus-leukemia effect due to the higher CsA concentration. Another explanation was the fact that the CsA300 group included significantly more patients with CML in the first chronic phase, the relapse rate of which is expected to be very low. Actually, the difference in the incidence of relapse became smaller when we excluded patients with CML. In addition, relapse in the CsA500 group mainly occurred in patients with relatively poor underlying diseases, including one with chronic neutrophilic leukemia in uncontrollable leukocytosis, one with acute myeloblastic leukemia with monosomy 7, and one with acute lymphoblastic leukemia with minimal residual disease detected by flow cytometry. Therefore, it might be important to make an appropriate definition of standard-risk disease. Currently, we are excluding acute leukemia in first remission with poor cytogenetic abnormalities, such as the presence of Philadelphia chromosome or monosomy 7, from standard-risk disease.

In conclusion, the continuous infusion of CsA with a target level of 450–550 ng/ml appeared to be safe and effective to prevent acute and chronic GVHD. A randomized controlled trial is being planned to confirm the appropriateness of this higher target level of CsA.

Patients and Methods

Patients

A continuous infusion of CsA with the target blood level between 450 and 550 ng/ml was started as GVHD prophylaxis for standard-risk patients at our institute in March 2003. We compared the safety and efficacy of this GVHD prophylaxis with those in the historical standard-risk patients in whom the blood CsA level was targeted to 250–350 ng/ml [6]. Standard-risk disease included acute leukemia in complete remission, CML in chronic phase, myelodysplastic syndrome without leukemic transformation, chemosensitive lymphoma, and nonmalignant disorders such as chronic active Epstein-Barr virus infection, while the others were considered high-risk diseases.

Transplantation procedure

Conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) with either busulfan (4 mg/kg/day for 4 days) or total body irradiation (TBI; 2 Gy twice daily for 3 days). BM was exclusively used as stem cell source in unrelated HSCT, whereas peripheral blood (PB) or BM was chosen in HSCT from a relative. GVHD prophylaxis consisted of CsA and short term methotrexate (MTX). The dose of MTX was 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6 in HLA-matched related HSCT. MTX at 7 mg/m^2 was added on day 11 in HLA-mismatched related HSCT and HLA-matched unrelated HSCT. In HLA allele-mismatched unrelated HSCT, the doses of MTX were increased to 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11.

CsA was administered as a 24-hr continuous infusion. The concentration of CsA was measured twice a week by fluorescence polarization immunoassay with a specific monoclonal antibody, using whole blood samples [20]. The dose of CsA was adjusted based on the ratio of the measured blood concentration and the target blood concentration of cyclosporine at 500 ng/ml to maintain the blood CsA concentration between 450 and 550 ng/ml. For example, when the measured blood concentration was 400 ng/ml using a daily cyclosporine dose of 200 mg, we multiplied the dose of cyclosporine by the ratio and determined the next cyclosporine dose at 200 mg × 500/400 = 250 mg. The route of CsA administration was converted to oral at a ratio of 1:2 when patients were able to tolerate oral intake after engraftment. Acute GVHD was graded as previously described [21]. Prophylaxis against bacterial, fungal, and *Pneumocystis carinii* infection consisted of fluconazole, tosufloxacin, and sulfamethoxazole/trimethoprim or inhalation of pentamidine. As prophylaxis against herpes simplex virus infection, acyclovir was given from days 7–35. Pre-emptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia. The initial dose of ganciclovir was 5 mg/kg once daily and the dose was elevated to 5 mg/kg twice daily, when an increasing antigenemia was observed [22]. Other supportive procedures were not changed.

Statistical considerations

Toxicities were evaluated until the route of CsA was changed to oral. Renal dysfunction was defined as elevation of serum creatinine level above ×1.5 or ×2.0 the baseline value. Liver dysfunction was defined as elevation of the total bilirubin level above 2 mg/dl. Dichotomous variables of the patients' characteristics in the two groups were compared using Fisher's exact test. Overall survival, disease-free survival, and the cumulative incidence of acute GVHD were calculated using the Kaplan–Meier method, whereas the cumulative incidences of relapse and nonrelapse mortality were calculated using Gray's method considering each other event as a competing risk [23]. Potential confounding factors for the analyses included age, sex, donor types (related or unrelated), stem cell sources (BM or PB), conditioning regimens (TBI or non-TBI), HLA-mismatch, total doses of MTX, and the target levels of CsA. To evaluate the influence of the confounding factors on these events, the log-rank test and proportional hazards modeling were used for univariate and multivariate analyses, respectively. Factors that showed at least borderline significance ($P < 0.10$) in univariate analyses were included in the multivariate analyses and stepwisely deleted from the model, although the target level of CsA was persistently stayed in the model. All P-values were two-sided and P-values of 0.05 or less were considered statistically significant.

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Case Report: Persistent Cytomegalovirus (CMV) Infection After Haploidentical Hematopoietic Stem Cell Transplantation Using In Vivo Alemtuzumab: Emergence of Resistant CMV Due to Mutations in the UL97 and UL54 Genes

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Addition of in vivo alemtuzumab to the conditioning regimen enabled 2- or 3-locus-mismatched hematopoietic stem cell transplantation with an acceptable incidence of graft-versus-host-disease. However, the procedure was associated with a high incidence of cytomegalovirus (CMV) reactivation. Although preemptive therapy with ganciclovir prevented successfully severe CMV diseases and CMV-related mortality, a patient developed persistent positive CMV antigenemia for more than 1 year after transplantation and CMV disease, despite the use of ganciclovir and foscarnet. The in vitro susceptibility assay showed that the clinical isolate was resistant to foscarnet, moderately resistant to ganciclovir, but sensitive to cidofovir. Therefore, cidofovir was administered. CMV antigenemia became negative within 2 weeks and never developed again. Nucleotide sequence of the UL54 and UL97 of the clinical isolate showed 4 amino acid substitutions (V11L, Q578H, S655L, and G874R) in UL54 and 2 mutations (A140V and A594V) in UL97 compared with the Towne and AD169 strains. Ganciclovir resistance was suspected to be caused by both A594V of UL97 and Q578H of UL54, whereas foscarnet resistance was due mainly to Q578H of UL54. In conclusion, the in vitro susceptibility assay as well as nucleotide sequence of clinical isolate is important to choose appropriate antiviral agents for patients who have persistent CMV reactivation after stem cell transplantation. ***J. Med. Virol. 80:1769–1775, 2008.***

KEY WORDS: CMV; resistance; mutation; hematopoietic stem cell transplantation; alemtuzumab

INTRODUCTION

Cytomegalovirus (CMV) disease is one of the major complications after allogeneic hematopoietic stem cell transplantation. Since the mortality rate is high, preemptive strategy by monitoring CMV reactivation with antigenemia assay or DNAemia assessed by the polymerase chain reaction have been developed for the management of CMV after hematopoietic stem cell transplantation [Boeckh et al., 1996; Kanda et al., 2002]. The risk factors for CMV disease after allogeneic hematopoietic stem cell transplantation include transplantation from an unrelated donor, the presence of HLA mismatch, the use of T-cell depletion, the development of acute graft-versus-host disease, the use of steroids, and so on [Nichols et al., 2001; Kanda et al., 2001a; Asano-Mori et al., 2005].

Alemtuzumab (Campath-1H) is a humanized monoclonal antibody directed against human CD52, that is

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expressed at a high density on B and T lymphocytes and dendritic cells, but not on hematopoietic stem cells [Gilleece and Dexter, 1993]. Therefore, the addition of alemtuzumab to the pretransplant conditioning regimen results in in vivo T-cell depletion and prevents acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation, even from HLA-mismatched donors [Kottaridis et al., 2000; Chakraverty et al., 2002; Kanda et al., 2005]. Although the use of alemtuzumab for in vivo T-cell depletion has been also reported to be a risk factor for CMV reactivation, preemptive therapy with ganciclovir or foscarnet can prevent an excessive incidence of CMV diseases and CMV-related mortality [Bainton et al., 2002; Nguyen et al., 2002; Laurenti et al., 2004; Kanda et al., 2005]. However, one patient developed persistent CMV antigenemia more than 1 year after haploidentical HLA-mismatched hematopoietic stem cell transplantation using in vivo alemtuzumab despite the use of ganciclovir and foscarnet. The clinical course of the patient and the results of the in vitro susceptibility assay and nucleotide sequence of the clinical isolate are described.

MATERIALS AND METHODS

Plaque Reduction Assay

The susceptibilities of the CMV isolate to ganciclovir, cidofovir, and foscarnet were examined by the plaque reduction assay [Cockley et al., 1988; Shiraki et al., 1990, 1991a,b; Yukawa et al., 1996]. Briefly, all assays were carried out in confluent HEL cell monolayers in 60 mm plastic dishes. The cells were infected with 100 plaque forming units (PFU)/0.2 ml of the CMV isolate and the Towne strain for 1 hr. Then, they were exposed to various concentrations of the drugs in 0.8% nutrient methylcellulose minimum essential medium supplemented with 2% fetal bovine serum, and incubated at 37°C as indicated below. The infected cells were fixed with neutral formalin and stained with methylene blue and then the number of plaques was counted. The inhibitory concentrations for 50% plaque reduction (IC_{50}) values were determined using the graph software MPM III-vs. 1.57 for Macintosh.

Sequence Determination of the UL57 and UL97 Genes

CMV DNA was prepared from the viral nucleocapsid as described previously [Shiraki et al., 1991; Ida et al., 1999; Yoshida et al., 2005]. The UL57 (DNA polymerase) and UL97 (phosphotransferase) genes were amplified by the primer sets of 5′-CCAACGAGCAGGCTTACC-3′ and 5′GTCGTCCTACGCGCGATACG-3′, and 5′-ACGCCTCTGTTCAGATTTTA-3′ and 5′-CCCACATGTAGATGGCGGCG-3′, respectively. The amplified fragments were sequenced by using ABI PRISM 3100 DNA sequencer according to the manufacture's procedures. The determined CMV UL54 and UL97 sequences were compared with the Towne (D14980 and U07355) and AD169 (X17403) strains and the nucleotide differences of the isolate common to the both were identified as the significant nucleotide changes.

Nucleotide Sequence Accession Numbers

The nucleotide sequences determined in this study have been deposited in the GenBank/DDBJ/EMBL database. The accession numbers of the CMV UL54 and UL97 genes from the patient were AB329634 and AB329635, respectively. The accession numbers of UL54 and UL97 used are D14980 and U07355 of the Towne strain and X17403 of the AD169 strain.

CASE REPORT

A 54-year-old man with myelodysplastic syndrome of refractory anemia with excess blasts (RAEB) with cytogenetic abnormalities including der(1;7)(q10;p10) participated in a clinical study of 2- or 3-locus- mismatched hematopoietic stem cell transplantation using in vivo alemtuzumab, because he did not have an available HLA-A/B/DR-matched related donor, 1-locus-mismatched related donor, or an HLA-matched unrelated donor. He had repeated episodes of infectious disease including pulmonary invasive aspergillosis due to sustained neutropenia. He was CMV-seropositive, but the donor was CMV-seronegative. The conditioning regimen consisted of alemtuzumab (0.2 mg/kg/day × 6 days, from day 8 to day 3), fludarabine (30 mg/m^2 × 6 days, from day 8 to day 3), busulfan (4 mg/kg/day × 2 days, from day 5 to day 4), and total body irradiation (2 Gy twice daily on day 1). Peripheral blood mononuclear cells were collected from his 3-locus-mismatched daughter, cryopreserved without ex vivo manipulation, and infused on day 0. The number of infused CD34 and CD3 positive cells was 5.24×10^6 cells/kg and 2.72×10^8 cells/kg of the recipient body weight, respectively. Post-transplantation prophylaxis against graft-versus-host disease was performed with 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). 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 12. Though febrile neutropenia was observed for 9 days, no bacterial and fungal infection was documented during neutropenia. Acute graft-versus-host disease was not clinically observed.

CMV reactivation was first detected on day 19 after transplantation by the CMV antigenemia assay that was performed weekly after engraftment using C10/C11 antibody. Preemptive antiviral treatment with intravenous ganciclovir (5 mg/kg twice daily) was initiated [Kanda et al., 2001b]. The level of antigenemia was decreased gradually and the dose of ganciclovir was decreased to 5 mg/kg/day. However, antigenemia increased again, which was refractory to the re-increase in the dose of ganciclovir to 5 mg/kg twice daily. Antiviral agents were changed from ganciclovir to foscarnet at 50 mg/kg twice daily adjusted to his renal function at day 72 after transplantation, that was temporarily effective but antigenemia increased again

within 1 month. Although the combination therapy of ganciclovir and foscarnet was started on day 125, it was also ineffective.

He developed CMV retinitis in his right eye with unilateral blurring on day 204. An ocular injection of 5 mg ganciclovir once a week was started and conducted four times. Then, retinitis of the right eye was well controlled. Otherwise, he did not have any symptoms of CMV diseases. Also, he did not have the clinical manifestations of acute or chronic graft-versus-host disease.

The in vitro analyses of the clinical isolate were conducted using his urine and peripheral blood which were collected on day 224 after transplantation as described in Materials and Methods Section. Table I shows the susceptibilities of the Towne strain and the clinical isolate to ganciclovir, cidofovir, and foscarnet determined by the plaque reduction assay. Clinical isolate was resistant to foscarnet with IC_{50} greater than 100 μg/ml, but moderately resistant to ganciclovir with IC_{50} at 4.54 μg/ml. The isolate was sensitive to cidofovir with IC_{50} at only 0.20 μg/ml, while the Towne strain was sensitive to all three antiviral agents. Therefore, the dose of ganciclovir was increased to 20 mg/kg/day. CMV antigenemia decreased gradually without any additional toxicities, but did not completely disappear. Finally, cidofovir was administered at 5 mg/kg/day three times, which resulted in the clearance of CMV antigenemia without recurrence for more than a year.

Although the number of lymphocytes, especially CD4+ and CD8+ T-cells, was strongly suppressed within 100 days after transplantation, the numbers of T-cells gradually recovered after day 100. The serum IgG level was persistently higher than 1,200 mg/dl after transplantation. The quantitation of CMV-specific cytotoxic T-cells was performed with the tetramer assay. HLA-A*2402 restricted CMV-specific cytotoxic T-cells were detected at 0.12% of CD8+ T cells on day 90 after transplantation, but not detected thereafter (Fig. 1).

Nucleotide sequences of UL54 and UL97 of the clinical isolate were compared with those of the Towne and AD169 strains. The nucleotide difference of the clinical isolate was determined by comparing the sequence difference common to both the Towne and AD169 strains. There were nucleotide differences of the clinical isolate of 49 and 46 (95 in total among 7,458 bases, 1.27%) in the UL54 and 15 and 20 (35 in total among 4,248 bases, 0.82%) in UL97 to the Towne and AD169 strains, respectively (Table IIA). The nucleotides identical to neither the Towne nor AD169 strain resulted in four and two amino acid substitution in UL54 (V11L, Q578H, S655L, and G874R) and UL97 (A140V and A594V) of the clinical isolate as shown in Table IIB. Nucleotide variations of the isolate from the Towne and AD169 were 0.3% for UL54 and 0.26% for UL97 and 28 and 7 of 35 nucleotide variations were transition and transversion, respectively. The nucleotide variation from AT to GC and GC to AT were 24 and 10 in comparison to the isolates with the Towne and AD169 strains and thus favored the shift from AT to GC. One (Q578H) of the four amino acid substitutions in UL54 has been known to be responsible for the resistance to ganciclovir and foscarnet (Fig. 2B). One (A594V) of the two amino acid substitutions in UL97 has also been reported before as being responsible for ganciclovir resistance (Fig. 2A).

TABLE I. IC_{50} (μM) of Towne and CMV Isolate to Ganciclovir, Cidofovir, and Foscarnet

	Towne	Clinical isolate
Ganciclovir	3.92 + 0.20	17.8 + 4.0
Cidofovir	0.39 + 0.18	0.72 + 0.18
Foscarnet	110 + 25	>794 (100 μg/ml)

The IC_{50} values were expressed as the mean + SEM of four independent experiments.

DISCUSSION

Preemptive therapy with ganciclovir has dramatically reduced the incidence of CMV disease after allogeneic hematopoietic stem cell transplantation. However, approximately one-fourth of patients experience an increase in positive cells by the CMV antigenemia assay (rising antigenemia) despite the use of ganciclovir [Nichols et al., 2001; Asano-Mori et al., 2005]. The use of steroid has been identified as the strongest risk factor for the development of rising antigenemia, and the in vitro antiviral susceptibility assay showed that most of the isolates were sensitive to ganciclovir. Therefore, the delayed immune recovery, not the resistant virus, might be the major cause of rising antigenemia. Transplantation from a CMV-seronegative donor might also have contributed to the delayed CMV immunity in the current patient [Nichols et al., 2001]. Nevertheless, the emergence of resistant strains is not uncommon, especially in patients who require prolonged use of antiviral agents. In hematopoietic stem cell transplant recipients, children with immunodeficiency syndromes, the use of T-cell depleted grafts, and the development of graft-versus-host disease have been reported to be risk factors for the emergence of resistant CMV strains [Eckle et al., 2000, 2002; Wolf et al., 2003]. In addition, it was reported that ganciclovir resistance emerged in two adult haploidentical hematopoietic stem cell transplant recipients after prolonged preemptive therapy [Wolf et al., 2003]. Therefore, the current patient was at very high-risk for the development of the resistant CMV strain.

The in vitro susceptibility assay showed that the clinical isolate was resistant to foscarnet, moderately resistant to ganciclovir, and fully sensitive to cidofovir. The results were compatible with the clinical course that cidofovir was far more effective than ganciclovir or foscarnet to terminate CMV antigenemia. To clarify the mechanism of resistance to ganciclovir and foscarnet, the nucleotide sequence of the UL97 and UL54 genes of the clinical isolate were determined. UL97 encodes a phosphotransferase that is required to phosphorylate ganciclovir to its triphosphate form with antiviral