Biology of Blood and Marrow Transplantation 13:1095-1105 (2007)

1083-8791/07/1309-0001$32.00/0
doi:10.1016/j.bbmt.2007.06.005

Randomized Comparison of Four-Times-Daily versus Once-Daily Intravenous Busulfan in Conditioning Therapy for Hematopoietic Cell Transplantation

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Received March 29, 2007; accepted June 7, 2007

ABSTRACT

Sixty patients were randomized to receive intravenous busulfan (iBU) either as 0.8 mg/kg, over 2 hours 4 times a day (BU4 arm) or 3.2 mg/kg, over 3 hours once a day (BU1 arm) in conditioning therapy for hematopoietic cell transplantation. The complete pharmacokinetic parameters for the first busulfan dose were obtained from all patients and were comparable between the 2 arms: for the BU4 and BU1 groups, elimination half-life (mean ± SD) was 2.75 ± 0.22 versus 2.83 ± 0.21 hours, estimated daily AUC was 6058.0 ± 1091.9 versus 6475.5 ± 1099.4 μM·min per day, and clearance was 2.05 ± 0.36 versus 1.91 ± 0.31 mL/min/kg, respectively. Times to engraftment after transplantation were similar between the 2 arms. No significant differences were evident in the occurrence of acute graft-versus-host disease (aGVHD) and hepatic veno-occlusion disease (VOD). Moreover, other toxicities observed within 100 days after transplantation were not significantly different between the 2 arms. The cumulative incidence of nonrelapse mortality was 20.8% in BU4 arm and 13.3% in BU1 arm. In conclusion, our randomized study demonstrates that the pharmacokinetic profiles and posttransplant complications are similar for once-daily iBU and traditional 4-times-daily iBU.

KEY WORDS

Intravenous busulfan • Once-daily administration • Conditioning therapy • Hematopoietic cell transplantation • Pharmacokinetics

INTRODUCTION

Busulfan, a bifunctional alkylating agent with potent toxicity against hematopoietic cells, is a common component of high-dose conditioning regimens for hematopoietic cell transplantation (HCT) [1-3]. Busulfan-based conditioning regimens are excellent alternatives to those involving total-body irradiation [4-8]. However, until recently, busulfan has only been available in the oral form. The efficacy of oral busulfan is frequently compromised by variable bioavailability and unpredictable systemic exposure over the course of treatment, largely because of the erratic absorption of oral agents and frequently complicated emesis [9,10]. Variations in systemic busulfan concentration may lead to overdosing, which has a higher risk of toxicity [9,11,12], or underdosing, which may result in graft failure or suboptimal antitumor activity [13,14]. In addition, intestinal absorption of busulfan may contribute to hepatic veno-occlusive disease (VOD) via a hepatic first-pass effect [15].

To avoid the inherent problems of oral administration and improve the accuracy of dosing, several formulations of intravenous busulfan have been developed in dimethyl sulfoxide [9], *N,N*-dimethylacetamide and polyethylene glycol 400 (Busulfex®, Orphan Medical, Minnetonka, MN) [16], liposomes [17], and microcrystalline lipids [18]. Busulfex®, the only currently available formulation, consists of a stable dissolved

drug that can be diluted further, either in normal saline or 5% dextrose in water, and delivered parenterally with 100% bioavailability [16]. A phase I trial showed that an intravenous busulfan dose of 0.8 mg/kg, yielded similar pharmacokinetic parameters to a standard oral dose of 1 mg/kg, [19]. The pharmacokinetic data were supported by a phase II trial in which patients with advanced hematologic malignant disease were treated with 16 doses of intravenous busulfan (0.8 mg/kg) every 6 hours, followed by 2 daily doses of intravenous cyclophosphamide (60 mg/kg) [20]. This regimen was also well tolerated, confirming the safety and efficacy of the drug at the dose. Moreover, intravenous busulfan displays a more consistent dosing and pharmacokinetic profile than oral busulfan. Parenteral busulfan formulations reduce hepatic sinusoidal exposure, decrease (but not altogether eliminate) interpatient variability in systemic exposure, and exclude dosing uncertainties associated with emesis. The 2-fold range of the area under the curve (AUC) following administration of intravenous busulfan is as narrow as that achievable by targeted oral dosing of busulfan plus therapeutic monitoring [11].

Although most alkylating agents are administered on a daily basis, in oral clinical trials, busulfan dosage was divided into 4 times per day to improve patient compliance, because of the sole availability of 2 mg tablets [21]. Consequently, most intravenous busulfan formulations have been administered as 4-times-daily dosing [22]. A once-daily dosage regimen of busulfan would be more convenient and tolerable than divided dosing. Preclinical data on busulfan treatment on a once-daily basis are available in animal models [23]. In children, busulfan was administered orally as a daily dose with no observed increase in toxicity [24]. Several single-arm studies additionally suggest that once-daily or twice-daily intravenous busulfan regimens are equally safe and effective, compared to the traditional 4-times-daily intravenous dosage [21,25,26].

In this study, we perform a prospective randomized trial of 4-times-daily versus once-daily dosing of intravenous busulfan (Busulfex®, Orphan Medical, Minnetonka, MN) in conditioning therapy for HCT. Pharmacokinetic characteristics of intravenous busulfan and clinical outcomes in terms of engraftment, complications, nonrelapse mortality, and overall survival are compared between the 2 administration schedules.

PATIENTS AND METHODS

Patients

Adult patients (15 years or older), who received intravenous 3.2 mg/kg/day busulfan on the first day of conditioning therapy for HCT, were included in this study. No other chemotherapeutic drugs were allowed on that day. A Karnofsky performance score of 70 or higher, and adequate cardiac, hepatic, and renal functions were required.

Protocol Outline and Randomization

This study was approved by the institutional review board of the Asan Medical Center. Patients provided informed consent before randomization. Patients were randomly assigned the 4-times-daily intravenous busulfan (BU4 arm) or once-daily intravenous busulfan (BU1 arm) treatment regimen. We employed the block randomization method using random number tables, including stratification according to the conditioning regimen (busulfan-cyclophosphamide [BuCy] versus busulfan-fludarabine-antithymocyte globulin [BuFluATG] versus busulfan only [Bu]). Busulfan was diluted in normal saline to 0.5 mg/ml, and infused by pump through a central venous catheter. Patients from the BU4 arm received intravenous busulfan (0.8 mg/kg) over 2 hours, 4 times a day, and those from the BU1 arm received it as 3.2 mg/kg over 3 hours once a day. All doses of busulfan were calculated using (1) actual body weight (ABW) if less than or equal to ideal body weight (IBW), (2) IBW if ABW was more than, but within 120% of IBW, or (3) "IBW + 0.40 * (ABW − IBW)" if ABW exceeded IBW by >120%.

For the BuCy regimen, intravenous busulfan (3.2 mg/kg/day) was administered on days −7 to −4 and cyclophosphamide (60 mg/kg/day) on days −3 and −2. The time between the last dose of busulfan and the first dose of cyclophosphamide was 14 hours in the BU4 arm and 27 hours in the BU1 arm. For the BuFluATG regimen, we administered intravenous busulfan (3.2 mg/kg/day) for 2 days (days −7 and −6), fludarabine (30 mg/kg) for 6 days (days −7 to −2), and antithymocyte globulin. Patients received 1 of 2 types of antithymocyte globulin according to availability in Korea, specifically, rabbit antithymocyte globulin (Thymoglobulin®, IMTIX-SANGSTAT, Lyon, France; 1.5 mg/kg/day on days −4 to −2 with a matched sibling donor, 3.0 mg/kg/day on days −4 to −2 with an unrelated donor, and 3.0 mg/kg/day on days −4 to −1 with a haplo-identical familial donor; n = 24) or horse antithymocyte globulin (Lymphoglobulin®, IMTIX-SANGSTAT, Lyon, France; 7.5 mg/kg/day on days −4 to −2 with a matched sibling donor, 15.0 mg/kg/day on days −4 to −2 with an unrelated donor, and 15.0 mg/kg/day on days −4 to −1 with a haplo-identical familial donor; n = 8). One patient received 20 mg alemtuzumab (MabCampath®, Schering, Berlin, Germany) on day −7, because of the unavailability of antithymocyte globulin in Korea at that time. For the Bu regimen, intravenous busulfan (3.2 mg/kg/day) was administered on days −6 to −3. The Bu regimen was employed specifically for autologous HCT.

Transplantation Procedure

All patients received an intravenous loading dose of phenytoin (15 mg/kg) the day before the first busulfan administration, and oral dosing was continued to maintain therapeutic levels (10 to 20 mg/L) until the day after the last dose of busulfan. Ciprofloxacin and acyclovir were administered for gut decontamination and viral prophylaxis, respectively. Hyperhydration and mesna were given to patients receiving the BuCy regimen for the prevention of cyclophosphamide-induced hemorrhagic cystitis. All cellular blood products were leukocyte-depleted and irradiated prior to transfusion. For patients undergoing allogeneic HCT, immunoglobulin (500 mg/kg) was administered intravenously on day −7, every other week until day 120, and monthly until day 180. Prophylactic therapy for graft-versus-host disease (GVHD) comprised cyclosporine alone in acute leukemia patients undergoing matched sibling donor HCT after conditioning treatment with the BuCy regimen, or cyclosporine plus methotrexate in other patients. Cyclosporine (1.5 mg/kg) was given intravenously every 12 hours starting on day −1, and then orally once oral intake became feasible. Intravenous methotrexate was administered at a dose of 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11. The day 11 dose was omitted for patients conditioned with the BuFluATG regimen for matched sibling donor HCT. Heparin was administered to patients receiving the BuCy or Bu regimen at a rate of 100 units/kg/day from day −7 to day 20, but discontinued in case clinically significant bleeding was observed or if the activated partial thromboplastin time (aPTT) exceeded the upper limit of control by 1.2 times.

Hematopoietic cell grafts were infused on day 0 (for bone marrow) or days 0 and 1 (for granulocyte-colony stimulating factor [G-CSF] mobilized peripheral mononuclear cells) without T cell depletion. All patients received intravenous G-CSF (450 μg, once daily), commencing on day 5, until the peripheral blood absolute neutrophil counts were over 3000/μL.

Monitoring of Patients

All patients were prospectively monitored for engraftment and posttransplant toxicities, including GVHD, hepatic VOD, and infections. Blood was withdrawn daily for complete counting, such as reticulocyte counts. Blood chemistry and electrolytes, including the magnesium level, were determined twice weekly or more frequently if necessary, whereas prothrombin time (PT) and aPTT were measured weekly.

The first day with an absolute neutrophil count ≥500/μL for 2 consecutive days was recorded for bone marrow engraftment. Patients who did not display absolute neutrophil counts of ≥500/μL for 2 consecutive days after HCT were categorized as "primary graft failure." Patients with initial engraftment in whom absolute neutrophil counts subsequently declined to <500/μL were classified as secondary graft failure (if they survived for at least 21 days). The first day of unsupported platelet counts ≥20,000/μL for 7 consecutive days was additionally recorded. Hematopoietic chimerism was evaluated in all allogeneic transplant patients, using peripheral blood samples from the donor and recipient, by PCR of short tandem repeats or amelogenin loci [27]. After transplantation, recipient peripheral blood samples were withdrawn monthly for the first 3 months, followed by every 3 months for an additional 1-2 years or until death. Complete donor chimerism was defined as the presence of only donor type hematopoietic cells after allogeneic HCT. Mixed chimerism was defined as the coexistence of both recipient and donor hematopoietic cells after allogeneic HCT. The degree of mixed chimerism was measured as the proportion of recipient cells in a given sample, and determined by the proportion of peak areas corresponding to recipient signals, compared to the sum of peak areas of donor and recipient signals.

Acute GVHD (aGVHD) was diagnosed on the basis of clinical symptoms, laboratory tests, and whenever possible, histopathologic findings of the skin, oral mucosa, and gastrointestinal tract [28], and classified according to clinical criteria [29]. Hepatic VOD was diagnosed in patients displaying at least 2 of the following before day 30: (1) hyperbilirubinemia (bilirubin ≥2.0 mg/dL), (2) painful hepatomegaly, and (3) unexplained weight gain (>2% from baseline) [30], with no other explanations for these signs and symptoms at the time of diagnosis. VOD was classified as mild, moderate, or severe [31]. Cytomegalovirus (CMV) infection was monitored weekly using shell vial culture [32] and CMV antigenemia assay [33,34]. A treatment schedule of 5 mg/kg ganciclovir every 12 hours was initiated in patients displaying CMV infection or disease.

Pharmacokinetic Studies

For pharmacokinetic studies, blood samples (5 mL) were obtained from all patients at 5 time points. This limited sampling strategy was adopted from a previous study [35]. Blood samples were obtained from patients in the BU4 arm at target time points of 2.5, 3, 4, 5, and 6 hours after the start of infusion, and 3.5, 5, 6, 7, and 22 hours after the start of the infusion in BU1 patients. Sampling was performed with the first dose only. Samples were taken from a peripheral vein into prechilled heparin tubes, with plasma separation within 30 minutes. Plasma was separated by centrifugation at 2500 rpm for 10 minutes at 4°C placed in cryogenic vials, and stored at −40°C until analysis by

validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) on the API 3000 triple quadruple mass spectrometer equipped with an electrospray ion source (MDS SCIEX, South San Francisco, CA). An aliquot of the sample (20 μL) was delivered into the electrospray ion source using HPLC (Agilent 1100 series, Agilent Technologies Inc., Santa Clara, CA) with a C_{18} Capcell Pak MG column (2.0 × 50 mm, 3.0 μm particle size). For validation procedures, plasma calibration curves, each consisting of 6 levels of busulfan (30-6000 ng/mL) and a fixed concentration of the internal standard (500 ng/mL, were prepared and assayed. To assess the intra- and interday precision and accuracy of the method, 5 replicates of the plasma standards at 3 concentrations (40, 400, and 4000 ng/mL) were analyzed. The calibration curves were linear throughout the concentration ranges examined, with correlation coefficients >0.998 for all cases. Based on a signal-to-noise level of 10, the limit of quantification for busulfan was found to be 30 ng/mL. Using the percentage deviation of the mean from the true value and the coefficient of variation (CV) as measures of accuracy and precision, respectively, the intra- and interday accuracy were determined to be 94.10-107.80% and 95.59-101.44%, respectively, and the intra- and interday precision were determined to be 3.82-6.29% and 2.42-10.14%, respectively, with 5 replicates at each concentration level.

The busulfan peak concentrations (C_{max}) are the observed values. Pharmacokinetic parameters, such as volume of distribution (V_d), half-life, and busulfan plasma clearance, were derived from a 1-compartment pharmacokinetic model. The AUC value per busulfan dose was calculated by dividing the drug dose by the busulfan plasma clearance estimate. The daily AUC value in the BU4 arm was estimated as 4-fold that of the AUC for the first dose, since previous studies consistently demonstrate that pharmacokinetic profiles of the first dose can be used as a predictor of later concentrations of the drug [21,25,26]. Pharmacokinetic modeling was performed using WinNonlin® v5.0.1 (Pharsight Corporation, Mountain View, CA).

Statistical Analysis

This was designed as a prospective, randomized, nonblind study. The primary endpoints of the study were the pharmacokinetic parameters, occurrence of grade III or higher toxicity within 100 days after HCT, and cumulative incidence of nonrelapse mortality. Toxicities observed within 100 days after HCT were graded according to NCI Common Toxicity Criteria v2.0, and classified between grades I and VI. Toxicities categorized as grades III to VI were recorded as severe. In addition, acute GVHD, CMV disease or infection, interstitial pneumonitis, and hepatic VOD were recorded separately.

Between-arm differences in baseline patient characteristics, occurrence of posttransplant toxicity, and pharmacokinetic parameters were compared by the chi-square test or Fisher's exact test for categoric variables, and Student *t*-test for continuous variables. This study included different conditioning regimens and we performed the Breslow-Day test to exclude heterogeneity of odds ratio for posttransplant toxicity in different conditioning regimens. If the test accepts the null hypothesis of the homogeneity of the odds ratio ($P \geq .05$), it is possible to compare the posttransplant toxicity by a single odds ratio using the chi-square or Fisher's exact tests. Cumulative incidence of engraftment and nonrelapse mortality were calculated and compared with Gray's method [36]. Overall survival was estimated with the Kaplan-Meier method, and compared using a long-rank test.

RESULTS

Patient Characteristics

In total, 60 patients were enrolled into the study between May 2004 and August 2005. We randomly assigned 30 patients to the 4-times-daily intravenous busulfan group (BU4 arm) and 30 to the once-daily group (BU1 arm). As of July 15, 2006, the median follow-up duration of surviving patients was 511 days (range: 299-736 days).

The distribution of patients was well balanced between the treatment groups with regard to patient and donor characteristics (Table 1), and transplantation procedure (Table 2). The median age of the 60 patients (23 women, 37 men) was 37.5 years (range: 16-58 years). Acute nonlymphoblastic leukemia, observed in 58% of the patients, was the most common indication for HCT. At the time of HCT, 17 patients (28.3%) displayed high-risk features, specifically, 10 with acute leukemia beyond first remission, 3 with chronic myeloid leukemia beyond the first chronic phase, and 4 with myelodysplastic syndrome having bone marrow blasts >5%. The median time from diagnosis to HCT was 185 days (range: 6-2399 days). All patients except 2, who received autologous hematopoietic cell grafts, underwent allogeneic HCT. The allogeneic hematopoietic cell graft donor was an HLA-matched sibling in 31 cases, a haploidentical family member in 6, and an unrelated volunteer in 21 patients. The median age of allogeneic donors was 36.0 years (range: 18-70 years). The donor-recipient sex pair was female to male in 15 (25.9%) cases, and 50% of the patients were ABO-mismatched with their donor.

The BuCy conditioning regimen was applied to 25 (41.7%), BuFluATG to 33 (55.0%) and Bu to 2 (3.3%)

patients. The immunosuppressive regimen for GVHD prophylaxis was cyclosporine alone in 15 (25.9%) and cyclosporine plus methotrexate in 43 (74.1%) patients. The hematopoietic cell graft was bone marrow in 36 (60.0%) and G-CSF mobilized peripheral mononuclear cells in 24 (40.0%) patients. The median mononuclear cell dose was 1.1×10^8/kg (range: 0.3-16.2 $\times$ 10^8/kg) and median $CD34^+$ cell dose was 5.3×10^6/kg (range: 1.0-125 $\times$ 10^6/kg).

Pharmacokinetic Parameters

A complete pharmacokinetic profile was obtained from all 60 patients. Parameters are shown in Table 3. The pharmacokinetic studies indicate that intravenous busulfan presents predictable linear kinetics with little variability in both arms (Figure 1). There were no significant differences between the BU4 and BU1 groups regarding V_d (mean [CV], 0.48 L/kg [12.5%] versus 0.46 L/kg [10.9%], $P = .187$), clearance (mean [CV], 2.05 mL/min/kg [17.6%] versus 1.90 mL/min/kg [16.3%], $P = .113$), and half-life (mean [CV] 2.75 hours [8.0%] versus 2.83 hours [7.4%], $P = .180$). Mean values of C_{max} and AUC of the BU1 arm (4217.6 ng/mL and 6475.5 μM·min) were about 4 times those of the BU4 arm (1064.6 ng/mL and 1514.5 μM·min). Thus, the estimated daily AUC value was equivalent between the BU4 and BU1 arms (mean [CV], 6058.0 μM·min [18.0%] versus 6475.5 μM·min [17.0%], $P = .145$). The range of estimated AUC values was 4179.3 to 8196.5 μM·min (median 5923.6 μM·min) in BU4 patients and 4347.0 to 8957.4 μM·min (median 6378.4 μM·min) in BU1 patients.

Table 1. *Patient and Donor Characteristics*

	BU4 arm (n = 30)	BU1 arm (n = 30)	P
Age, years			
35 or less	15 (50.0%)	14 (46.7%)	.796
>35	15 (50.0%)	16 (53.3%)	
Sex			
Male	17 (56.7%)	20 (66.7%)	.426
Female	13 (43.3%)	10 (33.3%)	
Diagnosis			
AML/acute mixed leukemia	16 (53.3%)	19 (63.3%)	.372
ALL	3 (10.0%)	3 (10.0%)	
CML	5 (16.7%)	3 (10.0%)	
MDS	3 (10.0%)	5 (16.7%)	
Miscellaneous	3 (10.0%)	0 (0.0%)	
Disease status at HCT			
Standard risk	23 (76.7%)	20 (66.7%)	.390
High risk	7 (23.3%)	10 (33.3%)	
Time from diagnosis to HCT, days			
180 or less	13 (43.3%)	15 (50.0%)	.605
>180	17 (56.7%)	15 (50.0%)	
Type of graft donor			
Autologous	1 (3.3%)	1 (3.3%)	.862
Matched sibling	15 (50.0%)	16 (53.3%)	
Haplo-identical familial	4 (13.3%)	2 (6.7%)	
Unrelated	10 (33.3%)	11 (36.7%)	
Donor age, years			
35 or less	15 (51.7%)	11 (37.9%)	.291
>35	14 (48.3%)	18 (62.1%)	
Sex pair (donor-recipient)			
Female-male	9 (31.0%)	6 (20.7%)	.368
Other	20 (69.0%)	23 (79.3%)	
Donor-recipient ABO incompatibility			
No	15 (51.7%)	14 (48.3%)	.793
Yes	14 (48.3%)	15 (51.7%)	

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; HCT, hematopoietic cell transplantation.

Table 2. *Transplantation Procedure*

	BU4 arm (n = 30)	BU1 arm (n = 30)	P
Conditioning regimen			
Busulfan-cyclophosphamide	12 (40.0%)	13 (43.3%)	.965
Busulfan-fludarabine-ATG	17 (56.7%)	16 (53.3%)	
Busulfan	1 (3.3%)	1 (3.3%)	
GVHD prophylaxis			
No	1 (3.3%)	1 (3.3%)	.667
Cyclosporine only	6 (20.0%)	9 (30.0%)	
Cyclosporine plus methotrexate	23 (76.7%)	20 (66.7%)	
Tissue for grafts			
Bone marrow	17 (56.7%)	19 (63.3%)	.598
Peripheral blood	13 (43.3%)	11 (36.7%)	
Mononuclear cell dose, $\times 10^8$/kg			
1.0 or less	12 (40.0%)	17 (56.7%)	.196
>1.0	18 (60.0%)	13 (43.3%)	
$CD34^+$ cell dose, $\times 10^6$/kg			
3.0 or less	6 (20.0%)	8 (26.7%)	.542
>3.0	24 (80.0%)	22 (73.3%)	

HCT indicates hematopoietic cell transplantation; GVHD, graft-versus-host disease; ATG, antithymocyte globulin.

Engraftment Data and Major Early Posttransplant Toxicities

No significant differences between the treatment groups were evident with regard to time to absolute neutrophil counts ≥500/μL, unsupported platelet

Table 3. *Pharmacokinetic Parameters Based on the Administration Schedule*

	BU4 arm (n = 30)	BU1 arm (n = 30)	P*
Volume of distribution (L/kg)	0.48 ± 0.06	0.46 ± 0.05	.187
Clearance (mL/min/kg)	2.05 ± 0.36	1.90 ± 0.31	.113
Half-life (hour)	2.75 ± 0.22	2.83 ± 0.21	.180
C_{max} (ng/mL)	1064.6 ± 147.2	4217.6 ± 700.1	<.001
AUC (μM·min)	1514.5 ± 273.0	6475.5 ± 1099.4	<.001
Estimated daily AUC (μM·min)	6058.0 ± 1091.9	6475.5 ± 1099.4	.145

AUC indicates area under the curve.
Mean ± SD.
*Student's *t*-test.

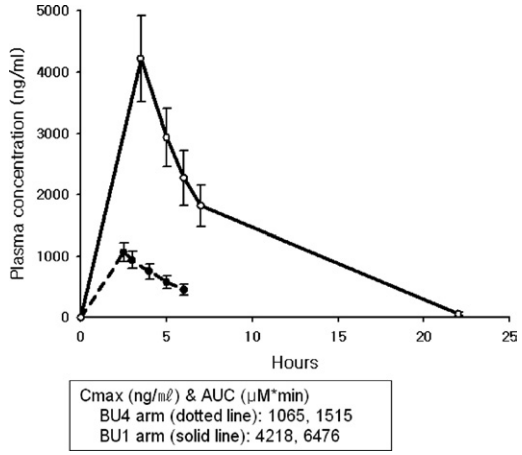

Figure 1. Busulfan concentration curves of the BU4 and BU1 groups.

counts ≥20,000/μL, and reticulocyte count ≥1%, occurrence of secondary graft failure, achievement of complete donor chimerism 4 weeks and 100 days after HCT, transfusion requirements within 100 days after HCT, and duration of G-CSF administration (Table 4). Cumulative incidence of acute GVHD among patients receiving allogeneic HCT was 31.0% in the BU4 arm and 13.8% in the BU1 arm (P = .145). CMV infection occurred in 12 BU4 (40.0%) and 7 (23.3%) BU1 (P = .165) patients, but did not develop to CMV disease. Three patients (10.0%) of the BU4 arm and 5 (16.7%) of the BU1 arm developed hepatic VOD (P = .448). No severe VOD was evident.

NCI Grading of Toxicities within 100 Days of Allogeneic BMT

Toxicity occurrence was investigated within 100 days after HCT, and graded according to NCI Common Toxicity Criteria v2.0 (Table 5). Severe pulmonary, renal, cardiovascular, and neurologic toxicity occurred infrequently on both arms, whereas severe metabolic abnormalities and infectious complications were frequently observed. Three patients in each arm developed severe neurologic toxicity, all of which occurred after 30 days posttransplantation. There were no significant differences between treatment groups with regard to the frequencies of severe toxicity, except nausea/vomiting and diarrhea. Specifically, the frequency of nausea/vomiting was higher (30.0% ver-

Table 4. *Engraftment Data and Major Early Posttransplant Toxicities*

	BU4 arm (n = 30)	BU1 arm (n = 30)	P
Engraftment			
Number of patients achieving ANC ≥500/μL	29	30	.313†
Median days to ANC ≥500/μL (range)	14 (10-24)	14 (10-29)	.872‡
Secondary graft failure	1	3	.317†
Number of patients achieving platelet ≥20,000/μL	26	26	1.000†
Median days to platelet ≥ 20,000/μL	26.5 (13-114)	25.5 (13-144)	.946‡
Number of patients achieving reticulocyte count ≥1%	23	27	.166†
Median days to reticulocyte count ≥1%	30 (13-602+)	26 (14-595+)	.216‡
Complete donor chimerism (%)			
4 weeks after HCT	25/27 (92.6%)	26/29 (89.7%)	.700†
100 days after HCT	22/23 (95.7%)	20/22 (90.9%)	.524†
Transfusion requirements within 100 days after HCT			
Red blood cells, units, mean ± SD	12.4 ± 9.6	11.8 ± 12.2	.845§
Platelets,* units, mean ± SD	124.0 ± 101.7	140.2 ± 146.1	.909§
Duration of G-CSF administration, days Mean ± SD	12.3 ± 4.2	13.4 ± 6.6	.443§
Acute GVHD	9/27	5/28	
Cumulative incidence	31.0%	13.8%	.145‡
Grade 1	2	0	
Grade 2	4	2	
Grade 3	2	0	
Grade 4	1	3	
CMV infection within 100 days after HCT	12 (40.0%)	7 (23.3%)	.165†
CMV disease	0	0	
Hepatic VOD	3 (10.0%)	5 (16.7%)	.448†
Mild	1	3	
Moderate	2	2	
Severe	0	0	

ANC indicares absolute neutrophil count; HCT, hematopoietic cell transplantation; SD, standard deviation; G-CSF, granulocyte colony stimulating factor; GVHD, graft-versus-host disease; VOD, veno-occlusive disease.

*One unit of single donor pheresis was calculated as 6 units of random donor platelet concentrates.

†Chi-square test.

‡Gray's method.

§Student's *t*-test.

Table 5. *NCI Grading of Toxicities within 100 Days after Allogeneic BMT*

	BU4 arm (n = 30)	BU1 arm (n = 30)	*P*
Pulmonary toxicities, grade III-IV	0	0	—
Hepatic toxicities, grade III-IV	13 (43.3%)	14 (46.7%)	.795*
Aspartate aminotransferase, increase	8 (26.7%)	5 (16.7%)	
Alanine aminotransferase, increase	12 (40.0%)	12 (40.0%)	
Bilirubin, increase	3 (10.0%)	5 (16.7%)	
Renal toxicities, grade III-IV	0	1 (3.3%)	1.000†
Renal failure	0	1 (3.3%)	
Coagulation abnormalities, grade III-IV	11 (36.7%)	6 (20.0%)	.152*
aPTT prolongation	3 (10.0%)	1 (3.3%)	
Disseminated intravascular coagulation	7 (23.3%)	6 (20.0%)	
Thrombotic microangiopathy	1	0	
Cardiovascular toxicities, grade III-IV	3 (10.0%)	1 (3.3%)	.301*
Arrhythmia	1 (3.3%)	0	
Hypertension	1 (3.3%)	1 (3.3%)	
Other	1 (3.3%)	0	
Gastrointestinal toxicities, grade III-IV	12 (40.0%)	9 (30.0%)	.417*
Stomatitis	4 (13.3%)	6 (20.0%)	
Nausea/vomiting	9 (30.0%)	0	
Diarrhea	0	4 (13.3%)	
Bleeding complications, grade III-IV	4 (13.3%)	6 (20.0%)	.488*
CNS bleeding	1 (3.3%)	0	
Epistaxis	1 (3.3%)	1 (3.3%)	
Gastrointestinal bleeding	2 (6.7%)	5 (16.7%)	
Hematuria	2 (6.7%)	0	
Metabolic abnormalities, grade III-IV	22 (73.3%)	22 (73.3%)	1.000*
Hypercalcemia	0	0	
Hyperglycemia	13 (43.3%)	10 (33.3%)	
Hypernatremia	0	1 (3.3%)	
Hyperkalemia	2 (6.7%)	2 (6.7%)	
Hypermagnesemia	1 (3.3%)	1 (3.3%)	
Hypocalcemia	2 (6.7%)	2 (6.7%)	
Hypophosphatemia	14 (46.7%)	10 (33.3%)	
Hypoglycemia	1 (3.3%)	0	
Hyponatremia	14 (46.7%)	9 (30.0%)	
Hypokalemia	13 (43.3%)	15 (50.0%)	
Hypomagnesemia	1 (3.3%)	2 (6.7%)	
Neurologic toxicities, grade III-IV	3 (10.0%)	3 (10.0%)	1.000†
Decrease of consciousness	3 (10.0%)	1 (3.3%)	
Seizure	0	1 (3.3%)	
Other	1 (3.3%)	2 (6.7%)	
Infectious complications, grade III-IV	23 (76.7%)	23 (76.7%)	1.000*
Febrile neutropenia	14 (46.7%)	13 (43.3%)	
Infection with neutropenia	18 (60.0%)	16 (53.3%)	
Infection without neutropenia	23 (76.7%)	22 (73.3%)	
Other	17 (56.7%)	17 (56.7%)	

Breslow-Day tests showed the homogeneity of the odds ratio for posttransplant toxicity between BuCy and BuFluATG regimens.
*Chi-square test.
†Fisher's exact test.

sus 0%, $P = .001$) and that of diarrhea was lower (0% versus 13.3%, $P = .038$) in the BU4 arm than the BU1 arm.

Nonrelapse Mortality and Overall Survival

Of the 60 patients analyzed, 23 deaths have been recorded (11 in the BU4 arm and 12 in the BU1 arm). Overall survival at 1 year was 69.0% in the BU4 arm and 70.0% in the BU1 arm ($P = .758$; Figrue 2). Eleven of the 23 deaths were related to relapse of underlying disease, whereas the remaining 12 were nonrelapse deaths (7 BU4 and 5 BU1 patients). Cumulative incidence of nonrelapse mortality at 2 years was 20.8% in the BU4 arm and 13.3% in the BU1 arm ($P = .488$; Figure 3). Nonrelapse deaths were caused by bleeding (n = 1; posttransplant day 24 [D24]), infection (n = 2; D41, D47), GVHD (n = 3; D62, D237, D335) and hepatic failure of unknown etiology (n = 1; D453) in the BU4 arm, and graft failure (n = 2; D60, D70) and GVHD (n = 3; D60, D62, D371) in the BU1 arm. Because all but 2 patients received 1 of the 2 conditioning regimens (BuCy or BuFluATG),

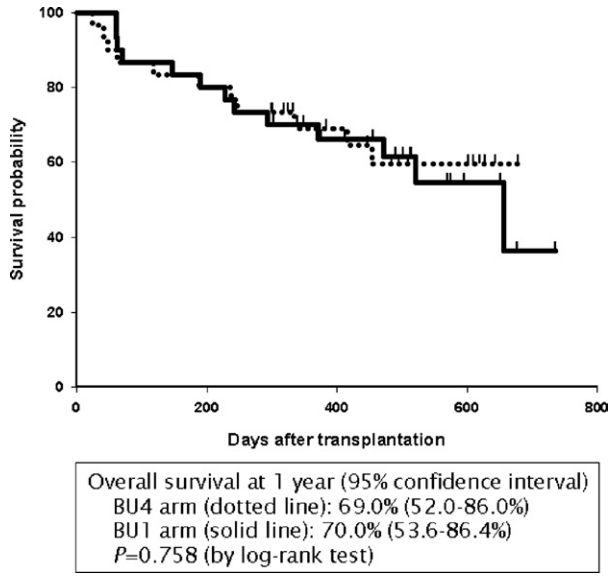

Figure 2. Overall survival of the BU4 and BU1 arms.

we performed subset analyses according to these regimens. Subset analyses revealed that nonrelapse mortality and overall survival were not significantly different between the BU1 and BU4 arms (Table 6).

DISCUSSION

Because busulfan is not cell cycle-nonspecific like other alkylating agents, frequent dosing or continuous infusion is not necessary to increase its effectiveness. Thus, it is reasonable to administer busulfan on a daily basis if there is no association with excessive toxicity. Several single-arm studies suggest that once-daily or twice-daily intravenous administration of high doses of busulfan do not increase posttransplant toxicity, compared to the traditional 4-times-daily schedule [21,25,26]. In our randomized trial, nonrelapse mortality and early posttransplant toxicities were not significantly different between the once-daily and 4-times-daily regimens of intravenous busulfan. There were 4 nonrelapse deaths among 30 patients in both arms within 100 days after HCT. Although 2 of the nonrelapse deaths in the once-daily arm were ascribed to secondary graft failure, no major differences in engraftment data were evident between the 2 schedules. Additionally, the cumulative incidence of acute GVHD was similar between the 2 treatment schedules. The frequency of hepatic VOD was low in both arms, and no severe VOD was observed. This finding was expected, because previous retrospective comparisons, including our experience, showed that use of intravenous instead of oral busulfan resulted in a lower frequency of hepatic VOD and related deaths [37-39]. The low incidence of hepatic VOD with intravenous busulfan may be because of avoidance of erratic absorption and hepatic first-pass effect of oral busulfan [12,37,39]. Severe posttransplant toxicities (defined as NCI grades III to IV) within 100 days after HCT were also comparable between the 2 administration schedules. Excessive exposure to busulfan may be associated with central nervous system toxicity [40]. In our study, severe neurologic toxicity was infrequent in both arms, and did not appear to be directly related to busulfan, because the time interval between the occurrence of severe neurologic toxicity and busulfan administration was >30 days. Phenytoin, administered to all patients during busulfan treatment, effectively prevented seizure development. Our results demonstrate that once-daily administration of intravenous busulfan, leading to a higher plasma peak concentration, has similar effects on engraftment of hematopoietic cells, and does not augment tissue injury to lung, kidney, heart, liver, and central nervous system after HCT, compared to traditional 4-times-daily administration.

Previous reports demonstrate that intravenous busulfan yields reproducible and predictable pharmacokinetics with less interdose and interpatient variability than oral busulfan [19,21,25,26]. The studies also suggest that once- or twice-daily administration of intravenous busulfan may yield similar pharmacokinetic parameters and toxicity profiles to the traditional 4-times-daily dosing. In a phase I study, the median AUC of busulfan in 5 patients who received intravenous busulfan dose of 0.8 mg/kg was 1132 μM·min (range: 964-1547 μM·min) and the AUC and other pharmacokinetic parameters at that dose were similar to those from oral busulfan dose of 1.0 mg/kg [19]. Intravenous busulfan (total daily dose of 3.2 mg/kg) was evaluated its safety and pharmacokinetics with a

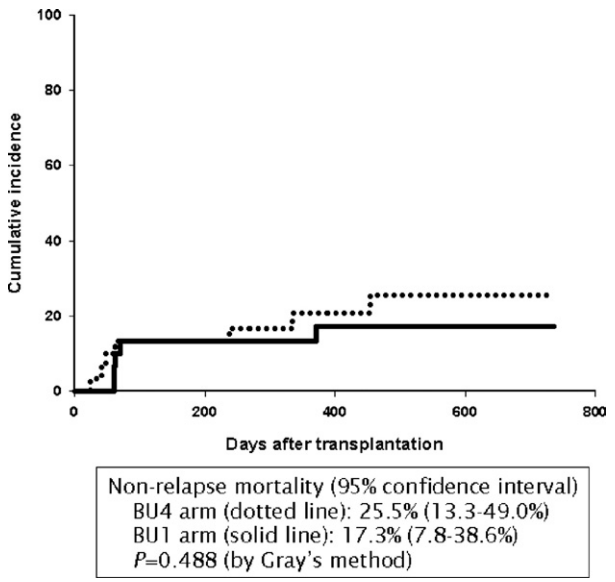

Figure 3. Nonrelapse mortality of patients in the BU4 and Bu1 treatment groups.

Table 6. *Nonrelapse Mortality and Overall Survival*

	Overall Survival*		Nonrelapse Mortality‡	
	100 Days†	1 Year†	100 Days†	2 Years†
All patients				
Bu4 arm (n = 30)	86.7% (74.5-98.9%)	69.0% (52.0-86.0%)	13.3% (5.4-33.2%)	20.8% (10.1-42.6%)
Bu1 arm (n = 30)	86.7% (74.5-98.9%)	70.0% (53.6-86.4%)	13.3% (5.4-33.2%)	13.3% (5.4-33.2%)
BuCy regimen				
Bu4 arm (n = 12)	83.3% (62.2-100%)	64.3% (35.7-92.9%)	16.7% (4.7-59.1%)	27.4% (10.3-73.0%)
Bu1 arm (n = 13)	92.3% (77.8-100%)	69.2% (44.1-94.3%)	7.7% (1.2-50.6%)	7.7% (1.2-50.6%)
BuFluATG regimen				
Bu4 arm (n = 17)	88.2% (729-100%)	70.6% (48.9-92.3%)	11.8% (3.2-43.3%)	17.7% (6.3-49.3%)
Bu1 arm (n = 16)	81.3% (62.2-100%)	75.0% (53.8-96.2%)	18.8% (6.8-52.0%)	18.8% (6.8-52.0%)

*Kaplan-Meier method, survival probability (95% confidence interval).
†Posttransplant.
‡Calculation of cumulative incidence, cumulative incidence (95% confidence interval); number of nonrelapse deaths: 7 in the Bu4 arm and 4 in the Bu1 arm.

twice-daily or once-daily schedule in a BuCy regimen. In the study, the median AUC of busulfan was 3390 μM·min (range: 2400-4678 μM·min) in 6 patients with twice-daily schedule and 5561 μM·min (range: 4414-7368 μM·min) in other 6 patients with once-daily schedule [21]. The study suggested that the change in dosing schedule did not increase toxicity or end-organ damage despite higher plasma concentration times. Two other studies investigated the safety of once-daily intravenous busulfan (3.2 mg/kg/day or 130 mg/m^2/day) plus fludarabine as conditioning for allogeneic HCT. The median AUC of busulfan was 4973 μM·min (range: 3432-6244 μM·min) with intravenous busulfan dose of 3.2 mg/kg/day (n = 12) [26] and was 4871 μM·min (range: 2931-8271 μM·min) with 130 mg/m^2/day dose (n = 45) [25]. Both studies showed that once-daily intravenous busulfan was well tolerated and did not increase toxicities. As evidence-based medicine requires randomized comparison and we designed a prospective randomized trial comparing once-daily versus traditional 4-times-daily intravenous busulfan. In our study, both once-daily and 4-times-daily administration of intravenous busulfan led to predictable linear kinetics with little interpatient variability, as reflected by a CV of 20% or less in all pharmacokinetic parameters, such as V_d, half-life, clearance, and estimated daily AUC. Moreover, these parameters were comparable between the 2 dosage schedules. The C_{max} the once-daily arm was about 4 times that of the 4-times-daily arm, as expected, but did not affect toxicity, at least in the concentration range of 0.8 to 3.2 mg/kg of intravenous busulfan. These findings are consistent with previous results, which disclose the importance of AUC or steady-state plasma concentrations, rather than peak concentrations, for posttransplant toxicity and effectiveness [11-14,21,25,26]. Our pharmacokinetic results clearly suggest that once-daily and traditional 4-times-daily intravenous busulfan are equally effective, in view of the comparable estimated daily AUC between the 2 schedules. Although our randomized study confirmed the safety of once-daily intravenous busulfan, further study for long-term efficacy should be performed. In our study, the estimated daily AUC was higher, although statistically not significant, in once-daily dosing than 4-times-daily dosing. The potential clinical impact of such differences should also be further investigated.

Once-daily administration of intravenous busulfan has several advantages over the traditional schedule. First, once-daily dosing is more tolerable and convenient for both carers and patients, compared to more frequent dosing. The chances of administration errors may also be lower. Second, less frequent administration of intravenous busulfan makes it possible to perform transplantation procedures in the outpatient setting. Third, our studies, in conjunction with other reports, show that intravenous busulfan (0.8-3.2 mg/kg) displays linear pharmacokinetics. Thus, a more consistent systemic exposure of intravenous busulfan is predicted with the once-daily dosage schedule because of no drug accumulation from 1 dose to the next. Fourth, single administration leads to a higher peak of busulfan concentration than divided dosing, and thus better penetration of poorly vascularized "sanctuary sites." Fifth, the once-daily schedule may have some theoretic advantages over 4-times-daily administration regarding hepatic VOD, since glutathione-S-reductase and glutathione-S-transferase recovery between doses is more viable with the once-daily approach [21]. Moreover, the time separation between the last dose of busulfan and first dose of cyclophosphamide in the BuCy regimen may contribute to a reduction in hepatic injury [9,14].

Pharmacokinetic studies on oral busulfan show that systemic drug exposure is related to clinical outcome after HCT. Low plasma busulfan concentration is associated with relapse of underlying disease [13] or graft rejection [41], whereas high plasma concentration is associated with the occurrence of hepatic VOD

[11,12] or nonrelapse mortality [42]. These findings have led to the development of individualized dose adjustment strategy [43], which targets the oral busulfan dose to the predetermined plasma level after a test dose. The low interdose variability of intravenous busulfan makes it more possible to adjust the concentration after a test dose or the first therapeutic dose, compared to oral busulfan [44]. Systemic exposure of intravenous busulfan is also related to posttransplant outcomes in patients with chronic myeloid leukemia [45]. However, considering the low interpatient variability of the pharmacokinetic parameters of intravenous busulfan, it is questionable whether more precise delivery of the appropriated quantity of intravenous busulfan by pharmacokinetics-guided individualized dosing would improve posttransplant outcomes (toxicities and/or effects). Our group and others [21,25,26] showed that the pharmacokinetic parameters of intravenous busulfan are within the 2-fold range, similar to that by targeted oral busulfan. The optimal AUC range for intravenous busulfan remains to be determined. Furthermore, a plasma busulfan concentration assay is not readily available in most clinical settings. It may be more feasible to identify the variables that have a significant influence on the pharmacokinetic parameters of intravenous busulfan, and develop an optimal dose calculation method to deliver more precise quantities of the drug. A pharmacogenomic approach may be additionally required [46].

In conclusion, our randomized study demonstrates that once-daily intravenous busulfan has similar pharmacokinetic profiles and posttransplant complications as the traditional 4-times-daily administered drug. In view of the theoretic advantages and convenience, we recommend the once-daily administration schedule for the use of intravenous busulfan as conditioning therapy prior to HCT.

ACKNOWLEDGMENTS

This study was supported by a grant (2005-199) from the Asan Institute for Life Sciences, Seoul, Korea.

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