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Favorable Outcome of Hematopoietic Stem Cell Transplantation Using a Targeted Once-Daily Intravenous Busulfan–Fludarabine–Etoposide Regimen in Pediatric and Infant Acute Lymphoblastic Leukemia Patients

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A B S T R A C T

Conditioning regimens for pediatric acute lymphoblastic leukemia (ALL) usually include total body irradiation (TBI), but TBI may result in serious sequelae. Busulfan and cyclophosphamide have been used as an alternative to TBI. Etoposide also has been widely used to enhance antileukemic effect. However, toxicities have been reported in some studies using busulfan, cyclophosphamide, and etoposide regimen. A reduced toxicity myeloablative regimen using busulfan and fludarabine showed promising results. Also, therapeutic drug monitoring (TDM) and administration of targeted doses of busulfan have been recommended to improve the outcome of hematopoietic stem cell transplantation (HSCT). In this study, we evaluated the outcome of HSCT using a targeted once-daily i.v. busulfan–fludarabine–etoposide (BuFluVP) regimen in pediatric and infant ALL. Busulfan (age $\geq$ 1 year, 120 mg/m^2; age $<$ 1 year, 80 mg/m^2) was administered once daily as the first dose on day −8, and a targeted dose of busulfan was used according to the TDM results on days −7 to −5. Forty-four patients were evaluated. Donor-type neutrophil engraftment was achieved in all patients. Veno-occlusive disease occurred in 7 patients (15.9%), but all patients were successfully treated. Cumulative incidence of treatment-related mortality and relapse were 9.1% and 9.9%, respectively. One-year overall survival and event-free survival rates of all patients were 86.2% and 83.8%, respectively. Twelve patients (27.3%) were infants at diagnosis, and their 1-year overall survival rate was 83.3%. Our study demonstrated that HSCT using a targeted once-daily i.v. BuFluVP regimen showed favorable outcomes and could be an option for HSCT in pediatric and infant ALL.

INTRODUCTION

Treatment outcomes in pediatric acute lymphoblastic leukemia (ALL) have dramatically improved, but some high-risk patients still suffer from poor outcomes. Hematopoietic stem cell transplantation (HSCT) can be a curative treatment option for these high-risk or relapsed patients [1-5]. The usual conditioning regimens for pediatric ALL include total body irradiation (TBI) [6-8], but TBI often causes serious sequelae, such as growth impairment, endocrinologic and metabolic problem, and secondary malignancies [9,10]. Busulfan-based conditioning regimens with cyclophosphamide have been used as an alternative to TBI-based regimens in many diseases, including pediatric ALL [11,12].

Etoposide has been widely used in HSCT for lymphoid and myeloid malignancy because of its antileukemic effect [13,14], and a conditioning regimen containing busulfan, cyclophosphamide, and etoposide was used in many studies including pediatric patients [6,15-18]. However, toxicities have been also reported in some studies using busulfan, cyclophosphamide, and etoposide conditioning regimens [19,20]. A reduced toxicity myeloablative regimen using

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busulfan and fludarabine showed promising results [21-24]. Thus, we used a conditioning regimen composed of busulfan, fludarabine, and etoposide (BuFluVP) to enhance antileukemic effect and to decrease the toxicity for pediatric ALL patients.

Therapeutic drug monitoring (TDM) of busulfan and administration of a targeted dose have been recommended to improve the clinical outcome of HSCT because of the narrow therapeutic range and highly variable pharmacokinetics of busulfan [25-30]. For these reasons, TDM and dose modification of busulfan were applied in our transplantation center since 2009. In this study, we evaluated the outcome of HSCT using a targeted once-daily i.v. BuFluVP conditioning regimen for pediatric and infant ALL.

METHODS

Study Population and Study Design

Forty-four patients were evaluated. We retrospectively studied patients who underwent HSCT using a targeted once-daily i.v. BuFluVP regimen at Seoul National University Children's Hospital from March 2009 to January 2014. This study was approved by the Institutional Review Board of the Seoul National University Hospital (H-1107-024-368), and 7 patients were enrolled in our phase I study, which was registered at www.clinicaltrials.gov (NCT01018446) [30].

We collected and analyzed data regarding engraftment, regimen-related toxicities, events, and survival. Events were defined as relapse or treatment-related mortality (TRM). TDM results were also analyzed. We analyzed infant leukemia separately, because infant leukemia is a specific group of diseases, and it is very difficult to apply TBI in this group of patients.

Transplantation Protocol

Donor selection was based on HLA serologic typing performed for class I antigens and HLA molecular typing for the DRB1 and DQB1 loci. HLA-A, -B, -C, -DRB1, and -DQB1 were confirmed by a high-resolution molecular method for all patients and unrelated donors. Suitable donors were selected in the order of matched sibling, unrelated donor, and cord blood.

The conditioning regimen was composed of busulfan, fludarabine (40 mg/m^2 once daily i.v. on days −8 to −3), and etoposide (20 mg/kg once daily i.v. on days −4 to −2). Busulfan (120 mg/m^2 for patients aged ≥ 1 year and 80 mg/m^2 for patients aged < 1 year) was administered once daily as the first dose on day −8, and a targeted dose of busulfan was used according to the TDM results on days −7 to −5 [30].

Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine plus prednisolone for related HSCT, cyclosporine plus mycophenolate mofetil for cord blood transplantation (CBT), or tacrolimus plus methotrexate for unrelated bone marrow transplantation (BMT)/peripheral blood stem cell transplantation (PBSCT). Veno-occlusive disease (VOD) and infection prophylaxis were administered according to our center's guidelines for HSCT [31]. Patients received lipo-prostaglandin E_1 (alprostadil, Eglandin; Welfide, Osaka, Japan) at a dose of 1 μg/kg/day through continuous infusion for prophylaxis of VOD with or without low-molecular-weight heparin (nadroparine calcium, Fraxiparine; GlaxoSmithKline, United Kingdom). Patients received ciprofloxacin, itraconazole, or micafungin and acyclovir as a prophylaxis for infection. Intravenous immune globulin (.5 g/kg/dose) was infused every 2 weeks until day 100 and then monthly until day 180. Sulfamethoxazole-trimethoprim was discontinued 3 days before HSCT and then restarted after engraftment.

Engraftment and Toxicities

Myelogenous engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count of .5 × 10^9/L, and platelet recovery was defined as the day the platelet count was 20 × 10^9/L without platelet transfusions. Bone marrow examination was done at 1, 3, and 6 months and 1 year after HSCT. Hematopoietic chimerism was evaluated by molecular analysis of short tandem repeat regions. Regimen-related toxicity until 42 days after transplantation was graded according to the National Cancer Institute Common Toxicity Criteria (v4.0).

TDM and Dose Adjustment

The analysis by HPLC (Symbiosis Pharma; Spark Holland, The Netherlands) with tandem mass spectrometry was based on our previously described method [30]. Blood samplings were taken through the Hickman catheter line, which was not used for busulfan infusion before administration, at 0, 1, 2, and 4 hours after the end of infusion. Area under the curve (AUC) and clearance were calculated by a 1-compartment model using WinNonlin 5.2.1 (Pharsight, Mountain View, CA).

Target AUC was initially set up as 18,125 to 20,000 μg·h/L/day (4415 to 4872 μmol·min/L/day), and the dose was adjusted when AUC was out of that range. We planned to perform TDM on the first and fourth days and the day when a dose adjustment more than 25% was needed according to the results of a previous study [25]. From June 2009, we made changes in our design because we observed frequent occurrence of toxicities. The target AUC was reduced to 18,000 to 19,000 μg·h/L/day (4384 to 4628 μmol·min/L/day), and we performed TDM and dose adjustment daily. Also, the target AUC on the fourth day was decided as (median value of the total target AUC−cumulative AUC during 3 days) μg·h/L/day [30]. In this study, decreased target AUC and daily TDM were applied to 40 patients.

Table 1
Clinical Characteristics and Transplantation Data (N = 44)

Characteristics	Value
Median age, yr (range)	9.7 (.6-22.2)
Gender	
Male	21 (47.7)
Female	23 (52.3)
Immunophenotype	
Precursor B cell ALL	31 (70.5)
Precursor T cell ALL	8 (18.2)
ALL with biphenotype (B cell lymphoid and myeloid)	4 (9.1)
ALL with biphenotype (B and T cell lymphoid)	1 (2.3)
Transplant type	
Related BMT/PBSCT	10 (22.7)
Unrelated BMT/PBSCT	24 (54.5)
CBT	10 (22.7)
Pre-HSCT status	
First CR with poor prognostic factor	28 (63.6)
Second CR	12 (27.3)
Third CR, persistence or other*	4 (9.1)

Values are number of cases with percents in parentheses, unless otherwise noted.
* Reappearance of molecular (fluorescein in situ hybridization) marker.

Statistics

Differences between means in continuous variables were calculated with Student's *t*-test. Kaplan-Meier method and log-rank univariate comparisons were used to estimate survival. Cumulative incidence was calculated using a competing risk model. STATA version 13.0 (Stata Corporation, College Station, TX) was used for all statistical analyses, and statistical significance was accepted when $P < .05$.

RESULTS

Characteristics of Patients

The clinical characteristics of the patients are summarized in Table 1. Twenty-eight patients underwent HSCT in first complete remission (CR) because of poor prognostic factors (8 infant leukemia, 5 initial WBC > 200,000/μL, 4 ALL with biphenotype, 3 induction failure, 3 *MLL* positive, 2 *BCR/ABL* positive, 1 early T cell precursor leukemia, 1 hypodiploidy, and 1 infant *BCR/ABL* positive). Twelve patients (27.3%) were in second CR, 1 (2.3%) in third CR, and 2 (4.5%) in persistence at the time of HSCT. One patient had reappearance of a molecular marker up to 4% by fluorescein in situ hybridization analysis.

Engraftment Data

Median numbers of infused total nucleated cells and CD34$^+$ cells were, respectively, 13.8 × 10^8/kg (5.7 to 52.6 × 10^8/kg) and 6.2 × 10^6/kg (.9 to 29.4 × 10^6/kg) in BMT/PBSCT and 9.8 × 10^7/kg (3.1 to 24.3 × 10^7/kg) and 3.8 × 10^5/kg (.5 to 5.9 × 10^5/kg) in CBT. Donor-type neutrophil engraftment was achieved in all patients. The median number of days required to reach an absolute neutrophil count of more than .5 × 10^9/L was 10 days (8 to 29 days). Spontaneous platelet recovery more than 20 × 10^9/L was achieved, except in 3 patients who

died before platelet engraftment and required a median 15 days (8 to 164 days).

SCT Complications

Elevation of aspartate and/or alanine aminotransferases or total bilirubin of at least grade 3 occurred in 24 (54.5%) and 3 patients (6.8%), respectively. Before the reduction of target AUC and daily TDM, aspartate and/or alanine aminotransferase elevation of at least grade 3 was observed in 4 patients, and 2 of them showed hyperbilirubinemia of at least grade 3. Among the 40 patients who underwent HSCT after the modification, 20 patients (50.0%) had elevated aspartate and/or alanine aminotransferases of at least grade 3, and hyperbilirubinemia of at least grade 3 occurred in 11 patients (27.5%).

Seven patients (15.9%) developed VOD (all moderate according to McDonald et al. [32]), and all were successfully treated. The total AUC of patients with VOD were significantly higher than total AUC of those without VOD (78,004 ± 5155 μg·h/L and 75,019 ± 2774 μg·h/L, respectively; $P = .030$). Septicemia occurred in 1 patient (2.2%) 6 days after HSCT.

Grades II to IV acute GVHD developed in 19 patients (grade II in 13 patients, grade III in 3 patients, and grade IV in 3 patients), with a cumulative incidence of 43.4%. Chronic GVHD developed in 7 patients, with a cumulative incidence of 16.1%.

Events and Survival Data

Four patients died of TRM, with a cumulative incidence of 9.1%. The causes of TRM were adenoviral pneumonia in 1 patient, respiratory syncytial viral pneumonia in 1, interstitial lung disease in 1, and infection with acute GVHD in 1 patient. Relapse occurred in 4 patients, with a cumulative incidence of 9.9%. Two were patients with precursor T cell ALL, and 1 patient was an infant who underwent HSCT in second CR because the patient showed very early relapse during consolidation treatment.

One-year overall survival (OS) and event-free survival (EFS) rates of all patients were 86.2% and 83.8%, respectively, with 25.8 months of median follow-up (Figure 1). EFS showed no difference according to the type of HSCT (80.0% in related BMT/PBSCT, 83.1% in unrelated BMT/PBSCT, and 77.1% in CBT, $P = .97$, Figure 2).

TDM Results

AUC of the first day ranged from 10,167 to 33,181 μg·h/L/day (median, 20,823 μg·h/L/day). In only 1 patient, AUC after the first day fell into the target range. Busulfan dose was increased on the second day in 13 patients, and a dose reduction was made in 30 patients. The total dose of busulfan ranged from 249.9 to 709.1 mg/m^2 (median, 391.6 mg/m^2), and the total AUC was 70,815 to 87,448 μg·h/L (median, 74,823 μg·h/L).

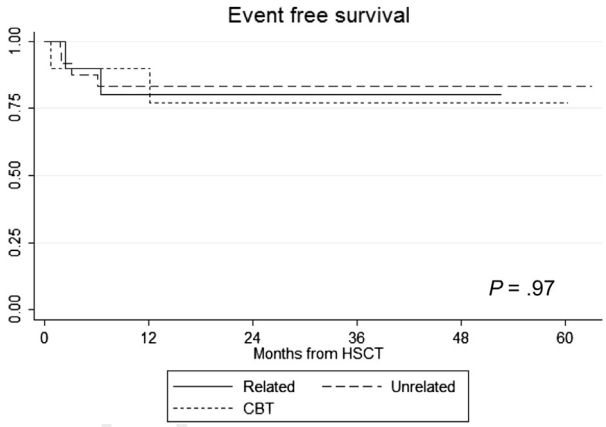

Figure 2. EFS showed no difference according to the type of stem cell transplantation (80.0% in related BMT/PBSCT, 83.1% in unrelated BMT/PBSCT, and 77.1% in CBT).

Infant ALL

In this study, 12 patients (27.3%) were infants at diagnosis, with a median age of .5 years (.1 to .9 years) (Table 2). Eight of these infants (66.7%) had *MLL* gene rearrangements and 1 had t(9;22). One patient who underwent HSCT in second CR relapsed at 2 months after HSCT, and 1 patient died of respiratory syncytial viral pneumonia at 1 month after HSCT. One patient who had persistent disease before HSCT achieved CR after HSCT and is alive without disease after 20 months of follow-up. The 1-year OS rate in these infant patients was 83.3%.

DISCUSSION

Conditioning regimens for pediatric ALL have been myeloablative regimens traditionally using TBI and high-dose cyclophosphamide [8]. Although TBI-based conditioning regimens have been widely suggested for pediatric ALL patients, long-term sequelae of TBI should be considered, especially in young children. In a report studying the late effects and health-related quality of life of childhood cancer survivors after radiotherapy, TBI was significantly associated with endocrine dysfunction [33]. Cardiopulmonary problems, severe cataracts, and secondary malignancies were also observed in other studies [7,34]. Children are usually in their growth and development period during treatment and also have a long life expectancy after HSCT. Long-term sequelae such as growth hormone deficiency, hypogonadism,

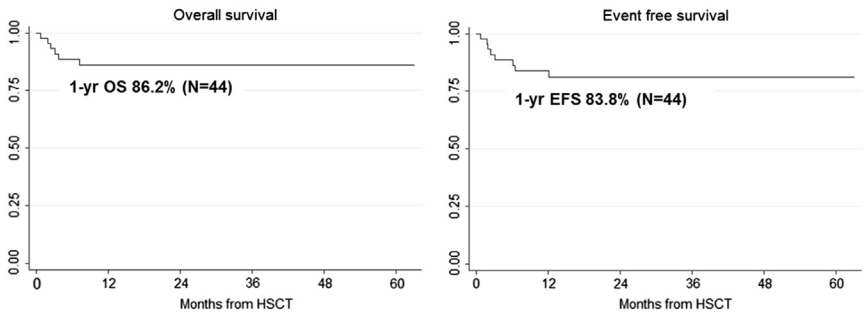

Figure 1. One-year OS and EFS rates of all patients were 86.2% and 83.8%, respectively.

Table 2
Infant ALL

Patient Number	Sex	Age at Diagnosis (yr)	Age at HSCT (yr)	Cytogenetics	Pre-HSCT Status	Type of HSCT	Status, Last Follow-up
5	F	.4	1.2	*MLL*	CR1	UPBSCT	NED, 59 mo
6	F	.7	1.8	del(9p)	CR1	UPBSCT	NED, 57 mo
7	M	.5	.9	*MLL*, t(4;11)	CR1	CBT	NED, 55 mo
8	F	.8	1.2	t(9;22)	CR1	RPBSCT	NED, 53 mo
11	F	.8	1.3	*MLL*	CR1	CBT	NED, 49 mo
17	F	.5	1.5	*MLL*, del(9p)	CR1	UPBSCT	NED, 33 mo
21	F	.1	.6	*MLL*, t(4;11)	CR2	UPBSCT	DOD, 4 mo
22	F	.4	1.3	*MLL*, t(11;19)	CR3	CBT	TRM, 1 mo
25	M	.8	1.3	del(9p)	CR1	CBT	NED, 27 mo
31	M	.3	.6		Persistence	RPBSCT	NED, 20 mo
32	F	.9	1.3	*MLL*	CR1	UPBSCT	NED, 19 mo
42	F	.2	.6	*MLL*, t(10;11)	CR1	CBT	NED, 7 mo

CR1 indicates first complete remission; UPBSCT, unrelated peripheral blood stem cell transplantation; NED, no evidence of disease; RPBSCT, related peripheral blood stem cell transplantation; DOD, dead of disease.

hypothyroidism, and secondary malignancy could be serious problems for children.

To avoid the late sequelae of TBI, conditioning regimens without TBI have been studied by some researchers [6,11,12]. A randomized trial comparing busulfan with TBI as a conditioning regimen for pediatric ALL [6] found similar relapses in both arms, but TRM was more frequent in busulfan arm, resulting in inferior EFS rates. However, a fixed dose of oral busulfan was used without TDM, and the authors suggested that targeting the level of busulfan could be an option to decrease TRM and improve outcome.

Busulfan has a narrow therapeutic range with high risk of toxicities such as VOD on high exposure [28,35-38] and increased relapse or graft failure on low exposure [28,39]. Because the pharmacokinetics of busulfan is known to be variable [29,40], TDM and dose adjustment of busulfan have been recommended to improve the outcome of HSCT [25-29]. In our previous reports, busulfan pharmacokinetics showed high inter- and intraindividual variability, and we suggested the need for intensive monitoring and dose adjustment of busulfan [30].

In this study, we performed TDM and dose modification of busulfan daily to reduce the effect of intraindividual variability and tried to meet the total target AUC by calculating the target AUC on the fourth day as a (median value of the total target AUC range—cumulative AUC during 3 days) μg·h/L/day. Many reports have shown that once-daily i.v. busulfan could be well tolerated as a conditioning regimen without increasing toxicity [41-44], and we used once-daily i.v. busulfan because of the convenience for TDM. We added etoposide (60 mg/kg) to enhance antileukemic effect and fludarabine instead of cyclophosphamide to reduce toxicities. With this targeted once-daily i.v. BuFluVP regimen, OS and EFS rates were 86.2% and 81.1%, respectively, and the cumulative incidence of TRM was 9.1%. These promising results suggest that once-daily i.v. BuFluVP with intensive TDM and dose modification could be an option for HSCT instead of a TBI-based regimen in pediatric ALL patients.

Unexpectedly, VOD still developed in 15.9% of patients even after this intensive TDM. This could be partly due to the addition of etoposide, because etoposide probably makes the conditioning regimen more toxic. Although VOD did not result in toxic death in this study, one should be aware of the possibility of VOD during the use of this regimen.

In our study, 10 patients (22.7%) underwent CBT, which is alternative means of HSCT in patients who do not have suitable siblings or unrelated matched donors. However, graft failure and early TRM are major obstacles to CBT. To enhance the engraftment potential, double-unit CBT has been attempted in many studies [45,46], but graft failure was still a problem. In this study, all CBT patients achieved neutrophil engraftment. One patient relapsed and 1 patient died of respiratory syncytial viral pneumonia. OS of these patients was comparable with that of patients who underwent related or unrelated BMT/PBSCT. Although the number of patients is not sufficient to draw any conclusion, optimization of the busulfan exposure by TDM could be one way to improve the outcome of CBT.

Twelve patients were infants at diagnosis, with a median age of .5 years. The outcome of infant leukemia is known to be very poor, with EFS rates of 42% to 47% 2 large studies [47,48]. There is insufficient evidence to support the benefit of HSCT in infant leukemia [49], but several studies have explored the use of HSCT to improve the outcome of infant leukemia, especially in cases of *MLL* positive [50-52]. However, TBI could result in serious sequelae, especially for these young patients. The outcome of HSCT of 12 infants in our study was promising, with an OS rate of 83.3%, considering many of them were carrying *MLL* gene rearrangement. This result suggests the feasibility of a targeted once-daily i.v. BuFluVP regimen to avoid severe toxicity and late sequelae in the patients with infant leukemia.

This study has its limitations in that it was a retrospective study with patients of a single institution. Also, some patients were not currently indicated for HSCT in centers in other countries. For example, HSCT of Ph^+ ALL is not routinely recommended in the United States and Europe after the Children's Oncology Group study [53] and EsPhALL trial [54], which showed excellent outcome of chemotherapy with imatinib. Chemotherapy with imatinib followed by HSCT has been a standard treatment for Ph^+ ALL in our center if there are matched donors. These factors should be considered in interpreting our data. However, our study showed tolerable toxicity and safety of targeted once-daily i.v. BuFluVP regimen. A future randomized multicenter trial is needed to confirm our results.

In conclusion, our study demonstrated that HSCT using a targeted once-daily i.v. BuFluVP regimen showed favorable outcomes in pediatric and infant ALL patients. The outcomes of HSCT were especially promising in infant ALL and CBT. With this result, a conditioning regimen of targeted once-daily i.v. BuFluVP could be one option for HSCT in pediatric and infant ALL patients.

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