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 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].

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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 (nadroparin 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) (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf).

TDM and Dose Adjustment

The analysis by HPLC (Symbiosis Pharma; Spark Holland, Emmen, 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.

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.

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.

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