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Highly Variable Pharmacokinetics of Once-Daily Intravenous Busulfan When Combined with Fludarabine in Pediatric Patients: Phase I Clinical Study for Determination of Optimal Once-Daily Busulfan Dose Using Pharmacokinetic Modeling

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Busulfan has a narrow therapeutic range, and in children, pharmacokinetic variability has been found to be high even after the use of intravenous (i.v.) busulfan. Recently, a reduced toxicity myeloablative regimen showed promising results, but the data of busulfan pharmacokinetics in hematopoietic stem cell transplantation (HSCT) using a targeted busulfan/fludarabine regimen in children has not yet been reported. We performed therapeutic drug monitoring (TDM) after once-daily i.v. busulfan combined with fludarabine and analyzed the outcomes. Busulfan (i.v.) was administered once daily for 4 consecutive days. The daily target area under the curve (AUC) was 18,125-20,000 μg*h/L/day (4415-4872 μmol*min/L/day), which was reduced to 18,000-19,000 μg*h/L/day (4384-4628 μmol*min/L/day) after a high incidence of toxicity was observed. A total of 24 patients were enrolled. After infusion of busulfan on the first day, patients showed AUC that ranged from 12,079 to 31,660 μg*h/L (2942 to 7712 μmol*min/L) (median 16,824 μg*h/L, percent coefficient of variation (%CV) = 26.5%), with clearance of 1.74-6.94 mL/min/kg (median 4.03 mL/min/kg). We performed daily TDM in 20 patients, and during the daily TDM, the actual AUC ranged from 73% to 146% of the target AUC, showing high intraindividual variability. The %CV of busulfan clearance of each individual ranged from 7.7% to 38.7%. The total dose of busulfan administered for 4 days ranged from 287.3 mg/m^2 to 689.3 mg/m^2. Graft failure occurred in 3 patients with total AUC less than 74,000 μg*h/L (18,026 μmol*min/L), and 2 patients with relatively high total AUC experienced veno-occlusive disease. Busulfan pharmacokinetics showed high inter- and intraindividual variability in HSCT using a targeted busulfan/fludarabine regimen, which indicates the need for intensive monitoring and dose adjustment to improve the outcome of HSCT. Currently, we are performing a newly designed phase II study to decrease regimen-related toxicities and reduce graft failure by setting an optimal target AUC based on this study.

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INTRODUCTION

Busulfan has a narrow therapeutic range. High exposure is associated with systemic toxicity such as veno-occlusive disease (VOD) [1-5], and underexposure is associated with graft failure or relapse [5,6]. After the intravenous (i.v.) formulation was introduced, busulfan pharmacokinetics appeared to be more predictable compared with the previous oral busulfan, especially in adults [7,8]. However, there is still a significant variation of busulfan exposure with the same i.v. dose, and a small proportion of patients will experience toxic exposure [9-12]. Because of this pharmacokinetic variability, therapeutic drug monitoring (TDM) of busulfan and dose adjustment have been recommended to improve

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the clinical outcome of hematopoietic stem cell transplantation (HSCT) [5,12-15].

Many recent reports have shown that once-daily i.v. busulfan could be well tolerated as a conditioning regimen without increasing toxicity [7,8,16,17]. One randomized study demonstrated that the pharmacokinetic profiles and posttransplantation complications are similar between once-daily i.v. busulfan and traditional 4-times-daily i.v. busulfan [18]. In 1 study, once-daily i.v. busulfan was also tolerable in children with limited toxicity, but the graft failure rate was relatively high; that indicated the need for optimization of the busulfan dose using TDM [19].

Recently, a reduced-toxicity myeloablative regimen using busulfan and fludarabine showed promising results [20-23], but the data of busulfan pharmacokinetics when combined with fludarabine in children has not yet been reported. In this study, we performed TDM after once-daily i.v. busulfan combined with fludarabine. We analyzed the pharmacokinetics of busulfan and also evaluated the clinical outcome of HSCT using a targeted busulfan/fludarabine regimen. We also analyzed the effect of a glutathione S-transferase (GST) polymorphism on the interindividual variability of busulfan pharmacokinetics.

MATERIALS AND METHODS

Study Population

Patients undergoing allogeneic HSCT using a busulfan-based conditioning regimen at Seoul National University Children's Hospital were prospectively included in this study from January 2009 to December 2009. This study was approved by the institutional review board of Seoul National University Hospital (H-0809-025-256) and registered at www.clinicaltrials.gov (NCT01018446). Written informed consents were obtained for all patients. During the study period, we decreased target area under the curve (AUC) after interim analysis of 13 patients, and we grouped the patients into group 1 (target AUC 18,125-20,000 μg*h/L/day) and group 2 (target AUC 18,000-19,000 μg*h/L/day).

Transplantation Protocol

The conditioning regimen was composed of busulfan and fludarabine (40 mg/m^2 once-daily i.v. on days −8∼−3). For patients with acute lymphoblastic leukemia, etoposide (20 mg/kg once-daily i.v. on days −4∼−2) was added. Busulfan (i.v.) was administered over 3 hours once daily for 4 consecutive days on days −6∼−3 for a busulfan/fludarabine regimen and on days −8∼−5 for a busulfan/fludarabine/etoposide regimen.

Busulfan was reported to have an age independent correlation between body surface area (BSA) and clearance in previous reports with children [19,24], so busulfan dosing based on the BSA was used in this study. Patients older than 1 year received 120 mg/m^2 as the first dose, and patients younger than 1 year received 80 mg/m^2. From the second day, we used a targeted dose of busulfan according to the TDM results. Bone marrow, mobilized peripheral blood, or cord blood was infused on day 0 of the conditioning regimen.

Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine plus prednisolone or mycophenolate, or tacrolimus plus methotrexate. Patients received low molecular weight heparin with lipo-PGE1 for prophylaxis of VOD. Other supportive care was according to the guidelines for stem cell transplantation in our center [25]. Regimen-related toxicity until 42 days after transplantation was graded according to the NCI Common Toxicity Criteria (NCI-CTC v4.0).

TDM and Dose Adjustment

A specific, accurate, and rapid assay based on high-performance liquid chromatography (Symbiosis Pharma, Spark Holland, the Netherlands) with tandem mass spectrometry was developed and validated for the quantification of busulfan in human plasma using glipizide as the internal standard. Human plasma samples were deproteinated using acetonitrile. Chromatographic separation for busulfan was performed on a Luna C18 column (5 μm, 100 Å, 50 mm × 2 mm; Phenomenex, Torrance, CA) with distilled water containing 0.1% formic acid-acetonitrile as the mobile phase by gradient elution. The flow rate was 0.3 mL/min, and the run time was 4.0 min. Busulfan and glipizide were detected using multiple reaction monitoring in the positive mode, with transitions of *m/z* 264.2 to 150.8 and *m/z* 446.3 to 321.1, respectively. Linear calibration curves were established in the range of 25-5000 ng/mL for busulfan, and the regression correlation coefficients (r) were over 0.9998. The intra- and interbatch accuracy values of quality control samples ranged from 96.5% to 100.4% and 98.5% to 99.9%, and precision variations of quality control samples were <4.3% and 4.7%, respectively.

Blood samplings were taken through the Hickman catheter line, which was not used for busulfan infusion before administration, and at 0, 1, 2, and 4 hours after the end of infusion. AUC and clearance was calculated by 2 compartmental methods using WinNonlin® 5.2.1 (Pharsight, Mountain View, CA). Target AUC was 18,125-20,000 μg*h/L/day (4415-4872 μmol*min/L/day), and dose adjustment was done when AUC was beyond the range. We initially planned to perform TDM on the first and fourth days, as well as the day when a dose adjustment of >25% was needed according to the results of previous study [13]. We changed the design to perform TDM daily, because the actual AUC at the fourth day was higher than the expected