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Busulfan Pharmacokinetics and Precision Dosing: Are Patients with Fanconi Anemia Different?

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It is well known that pharmacokinetics (PK)-guided busulfan (BU) dosing increases engraftment rates and lowers hepatotoxicity in patients undergoing hematopoietic cell transplantation (HCT). However, there are no published PK data in patients with Fanconi anemia (FA), who are known to have baseline DNA repair defect and related inherent sensitivity to chemotherapy. In our prospective, multi-institutional study of alternative donor HCT for FA using chemotherapy-only conditioning, we replaced the single dose of total-body irradiation with BU at initial doses of 0.8 to 1.0 mg/kg and 0.6 to 0.8 mg/kg given i.v. every 12 hours for 4 doses. Patients received the first dose of i.v. busulfan on day −8, and blood levels for PK were obtained. PK samples were drawn following completion of infusion. BU PK levels were collected at 2 hours, 2 hours and 15 minutes, and 4, 5, 6, and 8 hours from the start of infusion. The remaining 3 doses of BU were given on days −7 and −6. Thirty-seven patients with available BU PK data with a median age of 9.2 years (range, 4.3 to 44 years) are included in the final analyses. The overall BU PK profile in patients with FA is similar to non-FA patients after considering their body weight. In our cohort, a strong correlation between BU clearance and weight supports current practice of per kilogram dosing. However, not surprisingly, we show that the disease (ie, host) sensitivity related to FA is the main determinant of total dose of BU that can be safely administered to patients in this high-risk population. On the basis of our results, we propose an optimal BU concentration at steady-state level of ≤350 ng/mL (equivalent to total cumulative exposure of 16.4 mg*h/L for 4 doses over 2 days) for patients with FA undergoing HCT. To our knowledge, this is the first and largest report of prospective BU PK in patients with FA undergoing HCT, providing an optimal BU target cutoff to achieve stable donor engraftment while avoiding excessive toxicity.

INTRODUCTION

Busulfan (BU) is a bifunctional alkylating agent. In the setting of hematopoietic cell transplantation (HCT), given its narrow therapeutic index, BU is routinely dosed using therapeutic drug monitoring (TDM), with the goal of minimizing toxicity and decreasing graft rejection and relapse rates [1-5]. Precision dosing of BU is achieved by personalizing the BU dose to a target exposure, which is usually reflected by the measurement area under the plasma concentration-time curve (AUC) or concentration at steady state (Css). In adult and pediatric patients (eg, transplant for MDS (myelodysplastic syndrome /leukemia, cord blood transplants), BU pharmacokinetics (PK) has been studied extensively [6-10]. However, there are no published PK data in patients with Fanconi anemia (FA).

Patients with FA with their baseline DNA repair defect are inherently more sensitive to chemotherapy (especially DNA cross-linking agents) and radiation and remain at a higher risk for excessive toxicity [11-13]. Therefore, in general, significantly reduced doses of chemotherapy are needed in conditioning regimens for HCT for FA [14-19].

Patients with FA commonly also have short stature and/or failure to thrive. In addition, some may have kidney abnormalities, including single kidneys, crossed fused ectopia, and horseshoe kidneys with or without associated reflux nephropathy and reduced kidney function [20,21]. These baseline differences unique to patients with FA question the use of chemotherapy dosing extrapolated from the non-FA patient population.

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We and other groups have shown that radiation can be safely replaced with BU in the conditioning regimen of allogeneic HCT in patients with FA, including in the alternative donor setting [22-24]. This is specifically to avoid both short-term and long-term toxicity of radiation, including risk of solid tumors, given the increased baseline predisposition of squamous cell carcinoma of head and neck, genitourinary region, and other cancers in FA.

In our prospective, multi-institutional study of alternative donor HCT for FA using chemotherapy-only conditioning, we replaced the single dose of total-body irradiation with BU [22]. We hypothesized that BU at initial doses of 0.8 to 1.0 mg/kg and 0.6 to 0.8 mg/kg given i.v. every 12 hours for 4 doses would achieve adequate levels and ensure stable donor engraftment without excessive toxicity in patients with FA.

Reasons for choosing BU included the following: (1) at least a few published reports on the use of BU in patients with FA were available [25,26]. (2) An additional important factor was our ability to perform and understand BU PK and exposure in this high-risk population and administer PK-directed dosing to avoid excessive toxicity without an increase in graft rejection. (3) Prior experience at our centers with BU-based regimens allowed for initial selection of cutoffs for BU exposure.

MATERIALS AND METHODS

Conditioning Regimen

Conditioning regimen was as previously published and is shown in Figure 1. BU was given on day −8 (if PK samples were sent out) or on day −7 (if PK was performed in house) (PK with the first dose). On days −5 through −2, patients received daily fludarabine 35 mg/m^2/dose i.v., cyclophosphamide 10 mg/kg/dose i.v., and anti-thymocyte globulin (rabbit ATG) 2.5 mg/kg/dose i.v. A CD34$^+$ selected T cell depleted peripheral blood stem cell graft was infused on day 0. Immunosuppression for graft-versus-host disease prophylaxis consisted of cyclosporine starting on day −2 and continued through day +90 and then weaned over the next 10 weeks. Filgrastim was administered from day +1 and continued until the absolute neutrophil count was $>2 \times 10^9$/L for 3 days.

BU Level Assessment

Patients received the first dose of i.v. busulfan on day −8 (if PK samples were sent out) or on day −7 (if PK was performed in house) and blood levels for PK were obtained. BU was infusion was over 2 hours. PK samples were drawn following completion of infusion. BU PK levels were collected at 2 hours, 2 hours and 15 minutes, and 4, 5, 6, and 8 hours from the start of infusion. BU concentrations were measured by gas chromatography with mass spectrometry detection as previously described [27]. The within- and between-day coefficients of variation for the assay were below 8%. The dynamic range of the assay was from 125 to 7500 ng/mL with a lower limit of quantification of 125 ng/mL. PK analysis resulted on day −7 (afternoon), and these results were used to adjust (decrease only) the next 3 doses as required. The next 3 doses of busulfan were administered i.v. every 12 hours starting on the evening of day −7.

Planned Dose De-escalation for BU

There was a planned dose de-escalation for BU in the protocol. The first 19 patients received a starting planned dose of 0.8 to 1.0 mg/kg/dose i.v. every 12 hours (total 4 doses). The following 18 patients received a starting planned dose of 0.6 to 0.8 mg/kg/dose i.v. every 12 hours (total 4 doses) to potentially decrease toxicity. Standard age- and weight-based criteria were used to select the exact dose [4,9,28]. In the group that was assigned to receive the 0.6- to 0.8-mg/kg/dose, patients weighing <10 kg were assigned to receive 0.6 mg/kg/dose; patients weighing ≥10 kg but ≤4 years old received the 0.8-mg/kg/dose and patients >4 years old received the 0.6-mg/kg/dose. Similarly, in the standard-dose group, patients weighing <10 kg were assigned to receive the 0.8-mg/kg/dose; patients weighing ≥10 kg but ≤4 years old received a 1-mg/kg/dose and patients >4 years old received the 0.8-mg/kg/dose of BU.

Css Target and PK Analysis

A Css target of ≤450 ng/mL was initially chosen with the intent of limiting toxicity. The subsequent 3 doses of BU were reduced (if needed), based on PK results. BU concentration data were analyzed by standard noncompartmental analysis using Phoenix 64 WinNonlin software (Certara, Princeton, NJ), to obtain AUC, terminal half-life ($T_{1/2}$), and clearance (CL) values. Css estimate was calculated as AUC divided by the dosing interval [29] and, due to this observed trend, was same for both AUC/dose and Css/dose ratios. When or if the dose was changed after the first dose, AUC was linearly extrapolated based on AUC after the first dose using the dose ratio. Cumulative BU Css (ie, first dose and first dose Css + the estimated Css values from doses 2 to 4) was also correlated with toxicity (ie, hyperbilirubinemia, mucositis). Statistical difference was assessed with nonparametric comparisons for each pair using the Wilcoxon method. The analysis was performed using GraphPad Prism (version 7.04; GraphPad Software, La Jolla, CA). CL estimates were used to examine a correlation with body weight, age, and nuclear glomerular filtration rate (GFR) using GraphPad Prism.

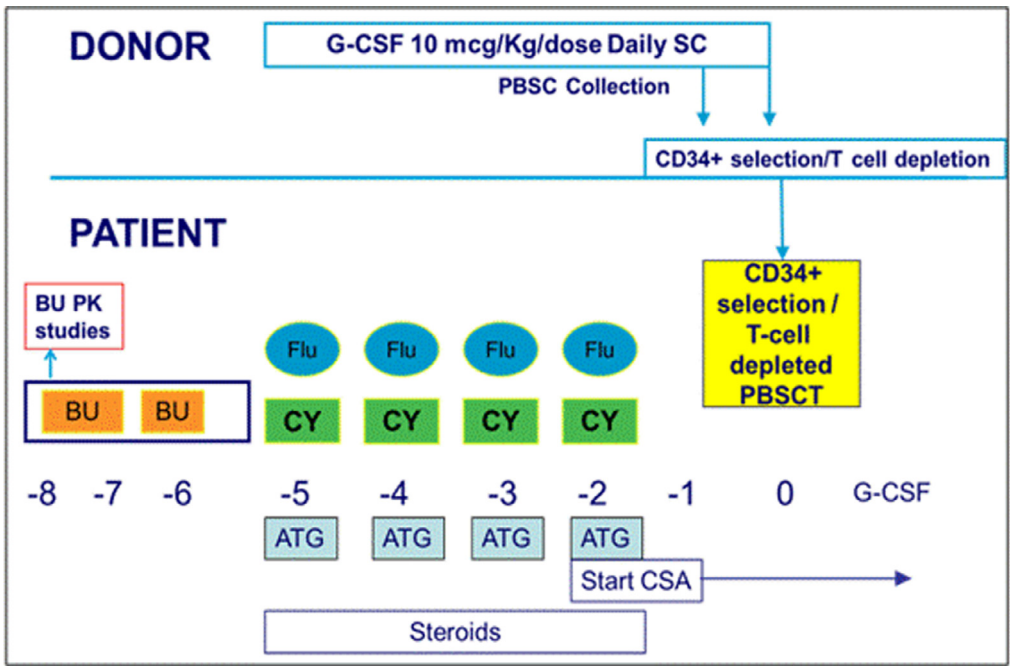

Figure 1. Conditioning regimen.

RESULTS

Patient characteristics and grouping based on BU dosing are described in Table 1. Thirty-seven patients with available BU PK data (from 2 centers) with a median age of 9.2 years (range, 4.3 to 44 years) underwent HCT between June 2009 and May 2014 and were included in the final analyses. Nineteen patients received standard-dose BU and 18 were in the dose de-escalated lower dose BU group. Regimen-related toxicities were graded according to the World Health Organization Toxicity Grading Scale (v3.0 and v4.0). All grade 3 to 5 severe adverse events (unexpected, definite, probable, and possibly related) were captured. Regimen-related toxicities were reported in detail previously [22]. Mucositis (n = 11 versus 21), hyperbilirubinemia (n = 3 versus 9), and hypertension (n = 3 versus 8) were less common ($P < .05$) in the lower BU dose group. Viral reactivation was the major infectious complication (n = 7 versus 12 in low-dose versus standard-dose group) and included adenovirus, BK virus, cytomegalovirus, and Epstein-Barr virus reactivation/infections, along with others. After 1 of the early patients developed sinusoidal obstruction syndrome (SOS), the BU exposure goal was lowered to a Css of ≤350 ng/mL (equivalent to total cumulative exposure of 16.4 mg*h/L for 4 doses over 2 days), and no further SOS occurred. Figure 2 shows the time-concentration profiles of all patients (Fig. 2A) and the median Css in the 2 groups after the first dose ($P = .0002$) (Fig. 2B). In the lower dose BU group, only 2 of 18 patients exceeded the Css of ≥350 ng/mL compared with 11 of 19 in the standard-dose BU group (Table 2). Percent dose adjustments were similar in both groups: 16.7% (n = 9) in the standard-dose BU group and 16.1% (n = 5) in the lower BU group ($P = NS$). Overall PK parameter estimates seen in both groups were comparable (Table 2). Median GFR was 112 mL/min/1.73 m^2 (range, 55 to 199 mL/min/1.73 m^2). BU CL correlated with body weight (kg) ($P < .001$) (Fig. 3), but the association between BU clearance and nuclear GFR was weak when normalized for body size (per 1.73 m^2; $P = .04$). Correlation of cumulative BU Css levels (Css for all 4 doses (first dose +3 additional doses) with toxicities did not show any significant correlation with hyperbilirubinemia ($P = .07$) or grade of mucositis ($P = .72$) in the pediatric cohort (age <18 years; n = 32). Rate of donor chimerism (≥95% of cells of donor origin without evidence of relapse) was similar in both groups (1 mosaic patient in the standard-dose BU group had a late graft failure).

DISCUSSION

To our knowledge, this is the first and largest report of prospective BU PK in patients with FA undergoing HCT. BU at the studied doses was well tolerated in patients with FA and generated effective levels to achieve our goals of stable donor engraftment while avoiding excessive toxicity (especially SOS). In addition, an optimal BU target Css level for HCT for patients with FA was defined, setting an important milestone in the field of HCT for patients with FA. Compared with the general population, patients with FA carry a 500-fold increased risk of developing squamous cell carcinoma of head and neck and genitourinary regions. Epidemiologic data suggest that radiation exposure and chronic graft-versus-host disease may increase risk of subsequent solid tumors [30-35], making safe use of PK-guided BU instead of total-body irradiation, as shown by our results, an important progress.

In a recent report by the Practice Guidelines Committee of the American Society of Blood or Marrow Transplantation, evidence-based review about personalizing BU-based conditioning confirmed that with the use of the standard myeloablative BU/cyclophosphamide regimen, PK-guided BU dosing increases engraftment rates in children and lowers hepatotoxicity in adults [1,3,29]. However, similar associations between BU exposure and outcomes were not found in patients receiving slightly different conditioning regimens [36,37]. This review also confirmed that usefulness of PK-directed dosing of BU in reduced-intensity conditioning (BU <9 mg/kg oral or i.v. equivalent) has not been systematically evaluated. Although

Table 1
Patient Demographics and Grouping Based on BU Dosing

Characteristics	Total Number/Median (range)	Standard Dose BU Number/Median (range)	Lower Dose BU Number/median (range)	P value§
Number of patients (from 2 centers)	37	19	18	-
Age in years	9.2 (4.3-44)	8.2 (4.3-31)	10.2 (4.3-44)	0.8747
Weight (kg)	26.4 (8.4-88.7)	26.4 (8.4-61.9)	25.4 (10.4-88.7)	0.8157
Nuclear GFR*	112 (55-199)	97 (67-199)	123 (55-150)	0.2118
Donor type - Related - Unrelated	 6 31	 5 14	 1 17	-

§Statistical difference was assessed by Mann-Whitney test.
*Nuclear GFR data were available for N=30 patients (N=16 for standard dose group; N=14 for lower dose group). Cr Cl was used in the remaining.

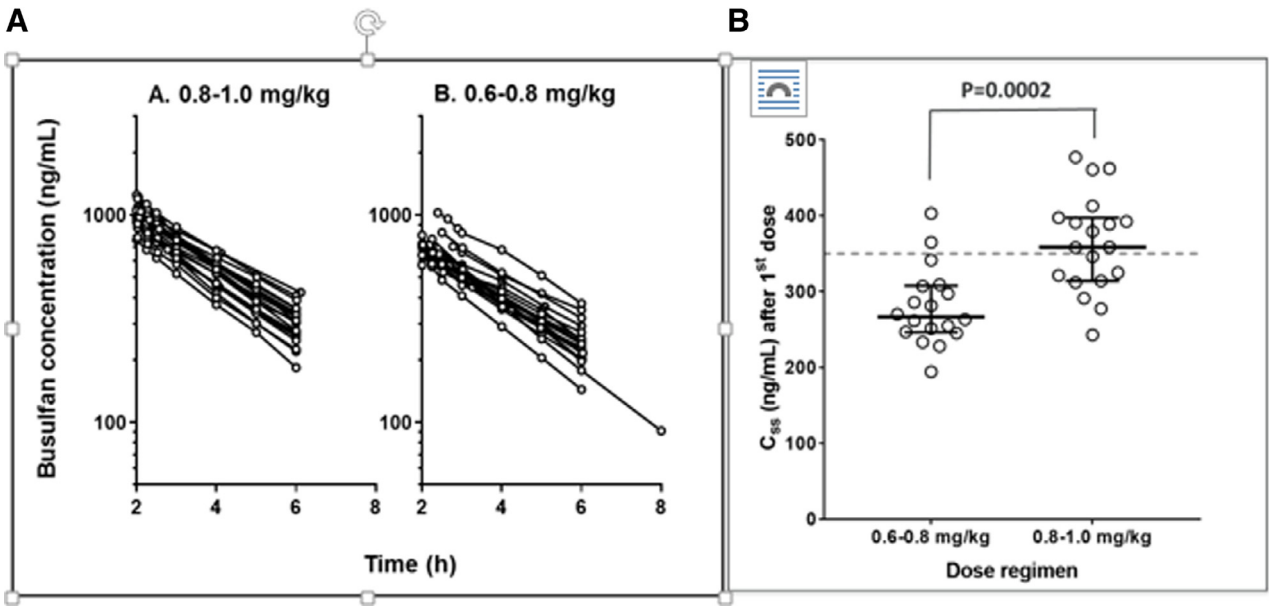

Figure 2. (A) Time-concentration profiles of all patients by 2 patient cohorts. (B) Median steady-state concentrations (Css) after the first dose of busulfan in the 2 patient cohorts.

Table 2
PK Parameters and Difference in the Steady-State Concentrations (Css) Achieved in the 2 Groups

Characteristics	Standard Dose BU Number/Median (range)	Lower Dose BU Number/median (range)	P value§
Number of patients	19	18	-
First dose (mg)	20 (6.5-49)	15 (6.0-52)	0.3162
C_{max} (ng/mL)	970 (758-1251)	708 (581-1026)	<0.0001
Half-life (hr)	2.46 (2.02-3.15)	2.64 (2.00-3.60)	0.5730
CL (L/hr/kg)	0.18 (0.14-0.28)	0.19 (0.12-0.24)	0.9578
CL (L/hr/70kg)#	9.81 (6.43-14.1)	9.93 (5.72-14.1)	0.9223
AUC (ng/mL*hr)	4305 (2917-5725)	3203 (2333-4840)	0.0002
C_{ss} (ng/mL)	359 (243-477)	267 (194-403)	0.0002
Number of patients with Css >350 ng/mL	11/19	2/18	-
Patient receiving a dose adjustment	9	5	-

§Statistical difference was assessed by Mann-Whitney test.

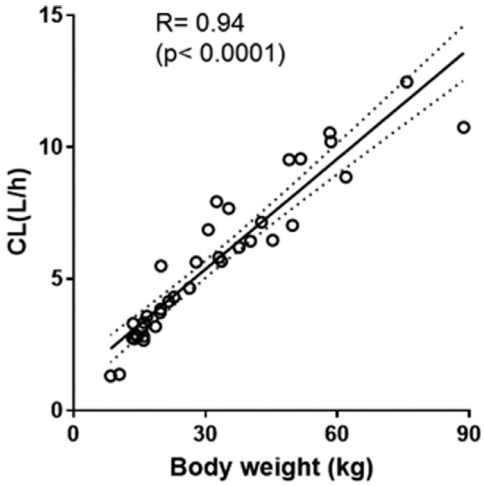

Figure 3. BU clearance (CL) correlates with body weight.

our regimen included total BU dose lower than 9 mg/kg, for patients with FA, this is considered a myeloablative conditioning, and risk of toxicity is high with higher exposure. More important, BU dosing recommendations in all of the studies above are in the setting of unmodified grafts. In contrast, in our study, we define a safe BU target level for the first time in the context of T cell depleted transplants.

Reducing toxicity is even more important in patients with FA given significant variability in weight and renal function abnormalities along with baseline chemotherapy sensitivity, due to the underlying defect in DNA repair. The overall BU PK profile in patients with FA is not different from non-FA patients after considering their body weight. In our cohort, a strong correlation between BU clearance and weight supports the current practice of per kilogram dosing. BU half-life in our cohort was very similar to a previously described half-life of 3.0 ± 0.7 hours in non-FA adults. In addition, clearance was also comparable to historical adult (0.15 L/h/kg) and pediatric (0.20 L/h/kg) norms in non-FA patients. Association between BU clearance and GFR (normalized for body size) was weak, suggesting only a small contribution of renal excretion to total BU clearance. However, not surprisingly, we show that the disease (ie, host) sensitivity related to FA is the main determinant of the total dose of BU that can be safely administered to patients in this high-risk population. On the basis of our results, we propose an optimal BU Css level of ≤350 ng/mL (equivalent to total cumulative exposure of 16.4 mg*h/L for 4 doses over 2 days) for patients with FA undergoing HCT. In comparison, optimal target Css for non-FA patients who receive full myeloablative BU doses (4 doses per day × 4 days) ranges between 817 and 1050 ng/mL [38].

BU PK results along with our clinical outcomes [22] have guided our currently open transplant study in which BU dose is adjusted based on age at HCT and disease status at transplant (marrow failure or MDS/leukemia), providing personalized therapy for patients with FA. Advances in the methods of PK-guided BU dosing, including the use of population PK model-informed Bayesian estimation and the identification of novel predictors of BU clearance such as metabolomics (eg, pharmacogenetics effect of glutathione S-transferase A1 enzyme), will further guide precision dosing of BU in other high-risk patients such as patients with FA.

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