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Pharmacokinetics of a Test Dose of Intravenous Busulfan Guide Dose Modifications to Achieve an Optimal Area Under the Curve of a Single Daily Dose of Intravenous Busulfan in Children Undergoing a Reduced-Intensity Conditioning Regimen with Hematopoietic Stem Cell Transplantation

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ABSTRACT

We studied 30 pediatric patients with malignant (n = 16) or nonmalignant (n = 14) conditions. The preparative regimen consisted of fludarabine, intravenous (IV) busulfan (Bu) for 2 daily doses, and antithymocyte globulin before stem cell transplantation. A test dose of IV Bu (0.8 mg/kg), anticipated to target an area under the concentration-time curve (AUC) of 800 to 1200 μmol · min, was followed later by 2 daily doses adjusted according to the pharmacokinetics (PK) to target an AUC of 3200 to 4800 μmol · min. The median test dose AUC was 953 μmol · min (range, 439-1315 μmol · min). The median AUC of single daily doses was 3798 μmol · min (range, 1511-7254 μmol · min). PK-based dose modification was required in 20 patients: 12 were adjusted to a higher dose, and in 8 the dose was decreased. Nausea and vomiting were noted in 15 patients. No patient developed hepatic veno-occlusive disease or seizures. Full donor chimerism was attained in 20 patients (mean of 24.5 days), 3 achieved partial chimerism, 5 did not engraft, and in 2 it is too early to assess chimerism. Acute graft-versus-host disease developed in 11 patients, grades I to II developed in 10 patients, and grade III developed in 1. Four patients died of infection and 5 of progressive disease. Thus, PK of a test dose of IV Bu provided information to adjust subsequent daily doses of IV Bu: this resulted in a regimen that was feasible, safe, and convenient for administration to children.

KEY WORDS

Single-dose daily regimen • Busulfan/fludarabine • Pediatrics • Pharmacokinetics • Hematopoietic stem cell transplantation

INTRODUCTION

The reproducible pharmacokinetic (PK) profile of intravenously (IV) administered busulfan (Bu) in adult patients undergoing stem cell transplantation (SCT) is in marked contrast to the erratic and unpredictable PK values noted with oral Bu in pediatric patients [1-3]. The erratic intestinal absorption of oral Bu and the variable clearance in pediatric patients contributes to wide intrapatient variability in area under the concentration-time curve (AUC) measurements [4,5]. With the use of the IV preparation, it is thought that this problem will be resolved. To evaluate the use of IV Bu in pediatric patients receiving single daily doses, we undertook a clinical trial to assess the PK of single daily doses in pediatric patients. We hypothesized that a test dose of IV Bu PK before transplantation allows for a strategy that could better predict a particular Bu target AUC in a patient receiving a single daily dose. The test dose of IV Bu could also result in less toxicity and better Bu exposure. There are no previously re-

ported clinical studies with single daily-dose IV Bu in children undergoing hematopoietic SCT (HSCT).

Most experience with the IV formulation of Bu has been with the traditional 4-times-daily dosing (16 doses) [6,7]. However, daily dosing and twice-daily dosing of IV Bu with a test dose have been found to be safe with reproducible PKs in patients with hematologic malignant diseases undergoing HSCT [8]. The collection of 4 or 5 samples after the first test dose was validated by demonstrating reproducible PK [8]. This finding confirms previous sampling strategies for pharmacokinetically directed dosing with high-dose IV Bu in HSCT preparative regimens [9]. It seems that a single daily dose of IV Bu × 4 days combined with cyclophosphamide can be used as a pretransplantation conditioning regimen for patients with advanced hematologic malignant disease [8].

The results of a recent study [10] demonstrated that a conditioning regimen of once-daily IV Bu 3.2 mg/kg for 4 days and fludarabine (Flu) 50 mg/m^2 for 5 days was relatively well tolerated and showed predictable Bu blood concentrations in 15- to 64-year-old patients undergoing allogeneic HSCT. Earlier trials with conditioning regimens used IV Bu in combination with cyclophosphamide because these myeloablative drugs were found to be successful in engrafting transplantation patients [6,11]. In these studies, IV Bu was administered 4 times daily for 4 days and produced predictable and consistent PK profiles along with acceptable toxicity [11].

In this study, a complete PK profile of a single daily dose of IV Bu was determined, along with the assessment of the value of the test dose to adjust the single daily dose to obtain an optimal AUC. The toxicity and engraftment of this combination regimen after transplantation were also assessed.

PATIENTS AND METHODS

Thirty consecutive pediatric patients with high-risk malignancies (n = 16) or non malignant (n = 14) conditions who were part of a reduced-intensity conditioning regimen (RIC) protocol underwent allogeneic HSCT with a single daily-dose IV Bu regimen at Children's Memorial Hospital from July 2003 to September 2005. Patient characteristics are summarized in Table 1. All patients were required to have their

Table 1. *Patient Characteristics*

Patient No.	Type of Donor	Degree of Matching	Age (y)	Diagnosis	Status of Disease at Transplantation
1	UCB	2-antigen mismatch	.5	SCID (Omenn)	Active disease
2	UCB	2-antigen mismatch	6	Neutroblastoma stage IV	PR2
3	MUD	10/10	16	ALL	CR2
4	MUD	9/10	8	CML	Molecular relapse
5	MUD	10/10	10	Infant ALL	CR1
6	MUD		3.5	Aplastic anemia	Unresponsive to immune therapy
7	UCB	2-antigen mismatch	.5	Infant leukemia	CR1
8	MUD	1-antigen mismatch	8	ANLL	CR2
9	MUD	9/10	6	Neuroblastoma stage IV	Persistent marrow disease
10	MUD	10/10	9	ALL	CR3
11	MUD	9/10	13	CML	Chronic 2
12	MS	10/10	6	Neuroblastoma stage IV	Relapse
13	MUD	10/10	10	ALL	CR2
14	MS	10/10	8	Hyper IgM syndrome	Active disease
15	PMR	8/10	.4	Gaucher type 2	Active disease
16	MUD	9/10	13	Rhabdomyosarcoma/MDS	Active disease
17	UCB	1-antigen mismatch	.1	SCID	Active disease
18	MUD	10/10	9	NHL in CR2	CR2
19	UCB	2-antigen mismatch	.4	SCID	Active disease
20	MS	10/10	10	ALL CR2	CR2
21	MUD	10/10	10	X-linked lymphoproliferative disease	Active disease
22	MS	10/10	14	NHL	Active disease
23	UCB	2-antigen mismatch	.7	SCID	Active disease
24	MS	10/10	15	CML	Chronic phase
25	MUD	9/10	1	Wiskott-Aldrich	Active disease
26	UCB	1-antigen mismatch	.4	SCID	Active disease
27	UCB	1-antigen mismatch	.1	Krabbe disease	Active disease
28	MUD	10/10	12	Kostmann	Active disease
29	MUD	10/10	1	Familial hemophagocytic lymphohistiocytosis	Active disease
30	MS	10/10	16	Aplastic anemia	Aplasia

UCB indicates unrelated umbilical cord blood; MUD, matched unrelated donor; MS, matched sibling; PMR, partially matched related; SCID, severe combined immunodeficiency; ANLL, acute nonlymphocytic leukemia; Ig, immunoglobulin; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; CR, complete remission; PR, partial response.

diagnosis confirmed by histological or laboratory testing. Signed informed consent by parents and assent of the patient (when applicable) to participate in the protocol and have their records used for research were obtained in compliance with the hospital's institutional review board.

Treatment Regimen

Patients were enrolled on an RIC transplant protocol developed at Children's Memorial Hospital [12,13]. The regimen included a single test dose (0.8 mg/kg) of IV Bu given over 2 hours on day −10, Flu 30 mg/m^2 on days −10 to −5, IV Bu 3.2 mg/kg/d on days −5 and −4 (as a 3-hour infusion with modifications of the dose based on the result of the test dose PK), and rabbit antithymocyte globulin (ATG) 2 mg/kg/d or equine ATG 40 mg/kg/d on days −4, −3, −2, and −1. Allogeneic hematopoietic stem cells were infused on day 0.

To optimize engraftment and minimize toxicities, it has become standard practice with regimens using 16 doses (IV or oral) to determine the PK of the first dose and adjust the remaining dosages. Because our regimen uses only 2 single daily doses of Bu, PK analysis of the initial single daily dose does not provide an opportunity to adjust the dosage to obtain an optimal AUC of Bu. Thus, we hypothesized that a test dose of IV Bu (0.8 mg/kg over 2 hours) several days before the 2 planned treatment doses of Bu could provide a PK basis for achieving an optimal AUC of Bu. PK analysis of the first treatment dose was performed to assess this hypothesis. A target AUC of 3200 to 4800 μmol · min was considered optimal for the single daily dose of Bu.

PK Determination of the Test and First Single Daily Dose

The collection of blood samples for PK of the test and single daily dose used in this RIC protocol were collected starting immediately before the start of the IV Bu infusion (baseline), and 15 minutes 1.0, 2.0, 2.5, 4.0 for the test dose, and 3 more samples at 8.0, 12.0, and 24 hours after the end of the infusion. The 24.0-hour sample was drawn before the start of the next infusion, on day −4. Blood samples were placed on wet ice after blood collection and processed within 1 hour after collection. Plasma was separated by centrifugation at 2500 rpm for 10 minutes at 4°C. Plasma was split into 2 equal amounts in 2 separate cryovials, labeled, and then stored at −20°C until the complete set of blood samples for each dose was obtained. It was then shipped to the Seattle Cancer Care Alliance Clinical Pharmacokinetics Laboratory (n = 28). For administrative reasons, the PK samples (test dose and first therapeutic dose) were assayed at the University of Pennsylvania pharmacology laboratory (n = 2) for analysis and determination of the AUC and clearance of IV Bu.

Calculation of the AUC, Clearance, and Dose-Modification Criteria

Clearance and bioavailability were calculated from the first dose by fitting a biexponential equation with the RSTRIP program (MicroMath, Salt Lake City, UT) to the data [14]. The AUC was calculated by trapezoidal approximation and extrapolation based on computer-generated parameters from 0 to infinity. The clearance was calculated by using the dose given divided by the weight and then divided by the AUC. On the basis of these parameters of the test dose, the dose was modified to achieve an optimal AUC for the single daily-dose administration.

Supportive Care

Patients underwent transplantation in the outpatient setting and received immune globulin (250 mg/kg) or CytoGam (Medimmune, Gaithersburg, MD) (100 mg/kg) on day −1 and continuing each week until day +100 and then monthly up to 6 months after transplantation or until they were able to sustain their immunoglobulin level without support. Phenytoin twice daily was administered for anticonvulsant prophylaxis on day −1 before single IV Bu was started with standard dosing for 48 hours after completion of IV Bu dosing.

Fluconazole 3 to 5 mg/kg/d IV or orally, with a maximum dose of 200 mg, was started with the conditioning therapy and continued until day +100. If systemic fungal infection was suspected or diagnosed after the completion of the preparative regimen, liposome amphotericin B (AmBisome, Fujisawa, Deerfield, IL) or voriconazole was instituted, and fluconazole was discontinued.

For the prevention of herpes simplex virus infections, acyclovir 250 mg/m^2 orally or IV was administered every 12 hours beginning on day −5 and continuing until day +100. Screening for cytomegalovirus was pursued with reverse transcriptase-polymerase chain reaction (PCR), which was performed weekly in patients at risk. If >1000 copies or an increasing number of copies were detected, ganciclovir was administered at 5 mg/kg every 12 hours until the number of copies was decreasing and was then administered at 5 mg/kg daily until 2 consecutive cytomegalovirus PCR assays became negative 1 week apart. To prevent *Pneumocystis carinii* infection, pentamidine 4 mg/kg IV was administered on day −1 and every 30 days up to 6 months after transplantation or 3 months after the cessation of immunosuppressive therapy.

Engraftment

We evaluated posttransplantation myeloid and T-cell chimerism weekly by using a rapid and sensitive

Table 2. *Busulfan Toxicities and Engraftment*

Patient No.	Seizures	VOD	N&V	Chimerism
1	No	No	Yes	Not achieved
2	No	No	Yes	14 d
3	No	No	Yes	21 d
4	No	No	Yes	Not achieved
5	No	No	Yes	53 d
6	No	No	Yes	19 d
7	No	No	Yes	15 d
8	No	No	Yes	27 d
9	No	No	Yes	39 d
10	No	No	No	21 d
11	No	No	No	Not achieved
12	No	No	Yes	21 d
13	No	No	Yes	21 d
14	No	No	Yes	21 d
15	No	No	Yes	14 d
16	No	No	Yes	Partial day 12 lost graft
17	No	No	No	61 d
18	No	No	No	27 d
19	No	No	No	14 d
20	No	No	No	14 d
21	No	No	No	35 d
22	No	No	No	29 d
23	No	No	No	Partial at 120 d
24	No	No	No	Not achieved
25	No	No	Mild	Partial at 42 d
26	No	No	No	Partial at 35 d
27	No	No	No	21 d
28	No	No	No	14 d
29	No	No	Mild	14 d
30	No	No	No	14 d

N&V indicates nausea and vomiting.

PCR method adopted at Children's Memorial Hospital. The method uses isolation of genomic DNA and RNA, synthesis of complementary DNA, PCR amplification, and DNA sequencing [15]. Donor chimerism after SCT was assessed with variable-number tandem repeat analysis of the peripheral blood.

Toxicities and Mortality

Regimen-related toxicities and transplant-related mortality were recorded. Death after relapse or progressive disease was designated as due to primary disease.

Statistical Analysis

Overall survival curves were produced by using the product-limit method of Kaplan and Meier [16]. To compare the measured AUC and the clearance of the test dose of IV Bu with the measured AUC and clearance of the single daily dose, a paired *t* test was used [17].

RESULTS

Patient Characteristics

Thirty pediatric patients were treated between July 2003 and September 2005. The patients' characteristics are summarized in Table 1. The mean age was 6.9 ± 5.4 years (SEM, range, 0.11-16 years): 17 male and 13 female patients were enrolled in the study. The median weight was 21 kg, with a range of 3.2 to 68 kg. Fourteen patients were diagnosed with nonmalignant conditions: aplastic anemia, hyper immunoglobulin M immunodeficiency, X-linked lymphoproliferative disease, Gaucher disease, Wiskott-Aldrich syndrome, Kostmann syndrome, Krabbe disease, Omenn syndrome, and severe combined immunodeficiency. Sixteen had malignancies: chronic myelogenous leukemia (CML), familial hemophagocytic lymphohistiocytosis, acute lymphoblastic leukemia (ALL), myelodysplastic syndrome, non-Hodgkin lymphoma, and neuroblastoma. There were 13 patients younger than 4 years of age.

Engraftment (Chimerism)

Twenty-one patients (70%) achieved full donor chimerism within a mean of 24.5 days after transplantation (range, 14-61 days; Table 2). Partial chimerism was noted in 2 patients (6 %) with graft loss at 12 and 160 days after initial engraftment. Four patients did not engraft, and 1 patient was not evaluable for engraftment because of an early death. In 2 patients it is too early to assess engraftment.

Regimen-Related Toxicity

None of the pediatric patients in this study developed hepatic veno-occlusive disease (VOD) or seizures. Mild to moderate nausea and vomiting were noted in 15 (50%) of 30 patients.

Acute graft-versus-host disease (GVHD) grade I to II was seen in 10 patients (33%), and 1 patient (3%) developed grade III GVHD after receiving a single daily-dose regimen of IV Bu Flu and ATG before allogeneic SCT (Table 3). No GVHD was found in the remaining evaluable patients (44%). No deaths were attributed to either acute or chronic GVHD.

Table 3. *Transplant-Related Toxicities*

Variable	n
Grades I-II acute GVHD	9
Grades III acute GVHD	1
No GVHD	20
Seizures	0
VOD	0
Nausea and vomiting	16 (mild to moderate)
Present status of patients	
Deceased due to infection	3
Deceased due to progressive disease	4
Alive and in clinical remission	19
Alive with disease	2
Second transplantation	1
Deceased from VOD after second transplantation (myeloablative)	1

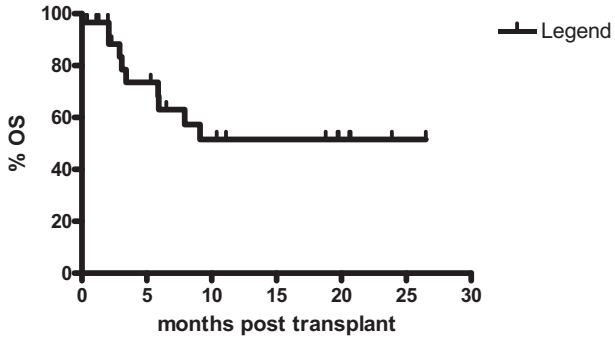

Figure 1. Kaplan-Meier plot of overall survival (OS) of pediatric patients.

Outcomes

Twenty-one (67%) of the children in the study are alive, and 19 were in clinical remission at a median of 18 months after transplantation (Figure 1). Transplant-related mortality occurred in 1 patient (3%), 2 patients developed infection (adenovirus and aspergillosis), and 5 patients (17%) developed disease progression (Table 3). Two patients not in remission received a second transplant, and 1 patient who received a second transplant subsequently died from VOD. A Kaplan-Meier plot of event-free survival after transplantation in pediatric patients showed nearly 60% survival of the patients who received a test dose and a reduced-intensity single daily dose of IV Bu Flu and an ATG conditioning regimen (Figure 1).

Table 4. *Summary of the Results of the Pharmacokinetics of the Test Dose and the Therapeutic Single Daily Dose*

Variable	Data
Test dose 0.8 mg/kg IV Bu	
Test dose administered total (mg)	**Mean, 20.3**
	Median, 18.8
	SD, 15.2
	Range, 3-68
Clearance (mL/min/kg)	**Mean, 3.6**
	Median, 3.5
	SD, 1.2
	Range, 2.4-7.3
AUC (μmol · min)	**Mean, 963**
	Median, 953
	SD, 215
	Range, 439-1315
Single-dose IV Bu administered (mg/kg)	**3.2 in 7 patients**
	>3.2 in 13 patients
	<3.2 in 10 patients
Actual single IV Bu dose administered (mg/kg)	**Mean, 3.4**
	Median, 3.2
	SD, 0.93
	Range, 2.5-7.2
Clearance (mL/min/kg)	**Mean, 3.6**
	Median, 3.5
	SD, 0.97
	Range, 2.5-6.9
AUC (μmol · min)	**Mean, 3740**
	Median, 3798
	SD, 1073
	Range, 1511-7254

Busulfan Pharmacokinetics

The results of the PK evaluation of a test dose of IV Bu 0.8 mg/kg are shown in Table 4. Samples from 26 patients yielded informative results. Results from 2 patients indicated incorrect timing of sample procurement, and 2 patients' samples were mishandled by the courier service during shipping. The total median test dose of IV Bu administered was 18.8 mg, with a range of 3 to 68 mg. The median clearance of Bu was 3.6 mL/min/kg (range, 2.4-7.3 mL/min/kg). The median AUC reached with the test dose in 24 of 30 patients was 953 μmol · min, with a range of 439 to 1315 μmol · min. Details of the PK results in 30 pediatric patients who received a single daily dose of IV Bu are shown in Table 4. The single daily dose of IV Bu administered was modified in 20 patients because of the results of the previously administered test dose. The scheduled dose of 3.2 mg/kg was administered to 8 of 30 patients, whereas lower adjusted doses ranging from 2.5 to 3.1 mg/kg were administered to 9 children. Upward adjustments were made in 13 children that ranged from 3.3 to 7.2 mg/kg. The actual median dose of IV Bu delivered was 3.2 mg/kg (range, 2.5 to 7.2 mg/kg). Median clearance was 3.5 mL/min/kg (range, 2.5-6.9 mL/min/kg). The median measured AUC was 3798 μmol · min (range, 1511-7254 μmol · min). The target AUC was achieved in 23 of 30 patients; 6 of the 7 patients who did not attain the target AUC were patients <2 years old.

Figure 2 shows the significant difference (P = .001) between the AUC achieved after a single test dose (0.8 mg/kg) of IV Bu versus a single daily dose (3.2 mg/kg) of IV Bu in pediatric patients, as expected with a 4-times-higher dose. As shown in Figure 3, the test dose clearance and the single daily-dose clearance were almost identical with the exception of 2 patients. One had a high clearance after the test dose, but it was

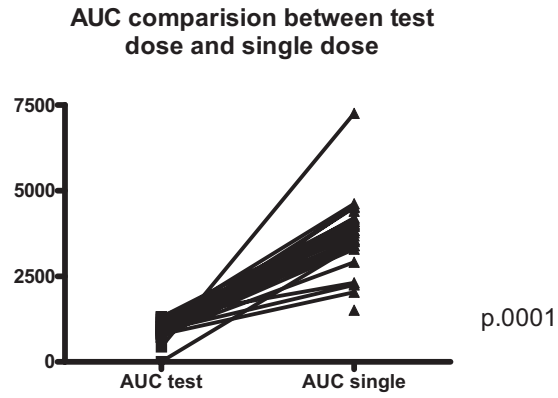

Figure 2. Comparative AUC between IV Bu test dose and single dose in pediatric patients.

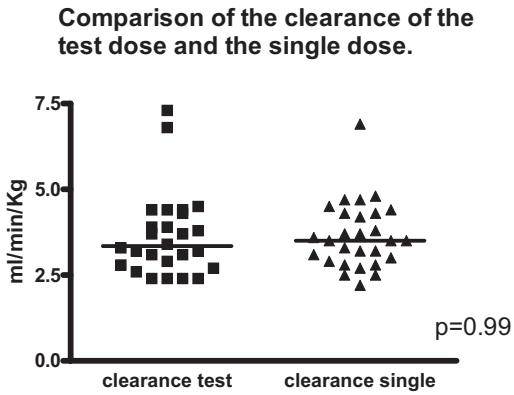

Figure 3. Comparison of the clearance of the test dose of IV Bu and a single IV dose in pediatric patients.

greatly reduced after the single daily dose. One patient with a normal clearance after the test dose had a much higher clearance after the single daily dose. Because no concomitant drugs were given during the Bu infusion, we cannot explain this discrepancy. There was no significant difference ($P = .90$) in clearance between the test dose and single daily dose. The patient distribution regarding dose modification and the achievement of an optimal AUC is shown in (Figure 4).

DISCUSSION

The chief finding of this study was that a test dose of IV Bu several days before the administration of a single daily dose was useful to adjust the doses of the single daily dose of Bu to achieve an optimal AUC in children >2 years of age. Another important finding of the study was that a regimen composed of Flu and 2 single daily-dose regimens (IV Bu and ATG) is a safe and effective conditioning drug combination in pediatric patients undergoing HSCT. Successful engraftment, as measured by full and mixed chimerism, occurred in 26 of 30 patients in the absence of VOD and other severe toxicities, which were not expected with this regimen. Favorable outcomes and low regimen-related toxicities in this study in children reflect similar outcomes in adult patients with high-risk and low-risk malignancies with a combination of IV Bu and Flu before allogeneic SCT.

One major problem using oral Bu in pediatric patients is nonretention of the drug as a result of vomiting and variations in bioavailability because of gastrointestinal permeability or intestinal metabolism [18-20]. The interpatient bioavailability of orally administered Bu in children varied as much as 5-fold [5], along with a 6- to 20-fold variation in apparent clearance [21-23]. An IV Bu formulation can avert these issues [6,24,25], although even with an IV formulation, variable clearance of Bu may result in either inadequate or excessive systemic drug exposure. Such PK and pharmacodynamic clearance possibilities with IV doses of Bu may increase the risk of graft rejection and disease recurrence or toxicity.

Therefore, in this study, a test dose of IV Bu was used in each pediatric patient before the administration of the conditioning regimens consisting of 2 single daily doses of Bu. We hypothesize that it is important to establish Bu clearance and AUC in each individual for a successful clinical outcome. An AUC of 800 to 1200 μmol · min of Bu was anticipated for the test dose. A median AUC of 953 μmol · min (range, 439-1315 μmol · min) was achieved, with a median clearance of 3.5 mL/min/kg (range, 2.4-7.3 mL/min/kg). These findings confirm the results of a previous study in which a test dose of 0.8 mg/kg resulted in a median AUC of 1034 μmol · min (range, 816-1315 μmol · min) and a median clearance of 3.1 mL/min/kg (range, 2.4-3.9 mL/min/kg) [26].

A test dose of IV Bu was necessary to determine whether modification of the single daily dose of IV Bu was required in each patient undergoing HSCT. The scheduled dose of 3.2 mg/kg was given only to 6 patients: the rest either had an upward or downward adjustment to achieve the desired AUC.

PK studies of IV Bu in children with malignant and nonmalignant conditions are limited. In one study of 6 children undergoing allogeneic HSCT and receiving IV Bu 4 times daily, systemic exposure of Bu was adequately estimated with limited sampling (2.5 and 4 hours). In a study of a small number of children, systemic exposure to IV Bu seemed to be relatively low in contrast to that in adults [27]. The low variability of IV Bu in children might be the result of the increased clearance previously described after the administration of oral Bu [28-30]. The total body clearance of Bu was significantly higher in young children (7.3 mL/min/kg) as compared with both older children and adults (3.02 and 2.7 mL/min/kg, respectively) [29]. A higher clearance of Bu in children than

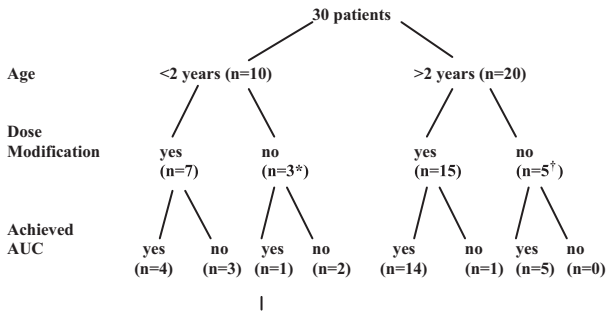

Figure 4. Patient distribution according to age, dose modification, and the ability to achieve an optimal AUC. *Two of these patients received the 3.2 mg/kg without results of a test dose only one achieved the desired AUC. †Two of these patients received the 3.2 mg/Kg without results of the test dose and both achieved the desired AUC.

in adults after IV Bu was confirmed in additional studies [8,31]. A significantly higher clearance of 3.61 and 3.79 mL/min/kg, respectively ($P < .005$), was observed in children after the first and the last dose of IV Bu compared with adults (2.40 and 2.33 mL/min/kg, respectively) [31].

Is important to note that despite adequate test dose PK attained, 6 patients did not achieve the target AUC, and 5 of 6 of these patients were <2 years old. It remains a challenging question to determine the optimal single daily dose of IV Bu in these patients. The recent retrospective review by Dalle et al. [32] reported that infants receiving IV Bu on a regular 6-hour schedule showed high interpatient variability. Therefore, it is important to consider careful evaluation of IV Bu PK in infants <1 year of age. A larger study will be necessary to explain the differences in IV Bu PK in younger patients (<2 years of age).

A PK study [21] showed that children with inherited disorders treated with oral Bu eliminated Bu significantly faster after the first and the last dose with half-lives of 1.93 and 1.71 hours, respectively, compared with children with leukemia (3.16 and 2.70 hours, respectively). The excretion of Bu in children after IV Bu was age and weight dependent [17], as found in previous studies [27,31]. Drug interactions may also influence the parameters of Bu PK: eg, the use of acetaminophen or itraconazole is known to affect liver enzymes [33].

In this study, the conditioning combination regimen of Flu and 2 single daily doses of IV Bu and ATG before SCT was found to cause mild nausea and vomiting and no VOD or severe mucositis. These results are similar to findings that were reported in a previous study that used IV Bu and Flu in patients 15 to 64 years of age with hematologic malignancies [10]. Transplantation-related mortality, an indication of acute toxicity, was relatively low, and the overall nonrelapse mortality rate indicates that a regimen of IV Bu and Flu did not seem to result in any major delayed engraftment [10].

We have previously reported that an RIC (nonmyeloablative) regimen consisting of Flu, IV Bu × 8 doses, and ATG for HSCT in children with nonmalignant diseases produced day +100 transplantation-related mortality of 15%, a 72% engraftment, and a 1-year overall survival of 84%, along with minimal short-term toxicities and an incidence of grade II to IV acute GVHD of 8% [12]. Therefore, it seems that an RIC regimen followed by SCT provides a good alternative to myeloablative SCT for children with nonmalignant disorders [12,35].

The relationship between the range of AUC values and toxicities with 2 single daily doses of IV Bu administration was confirmed in this study in children. That is, achieving targeted Bu AUC values with IV Bu was fundamental in the therapeutic value and in preventing the toxicities usually noted with the conditioning regimen [32,34-37].

In conclusion, Flu and 2 single daily doses of Bu and ATG as a conditioning regimen was effective and safe in 30 pediatric patients with malignant and nonmalignant conditions undergoing SCT. We found it useful to use a test IV Bu dose to determine whether it was necessary to adjust the single daily doses of IV Bu. PK studies showed that the clearance of the test dose correlated with the clearance of the therapeutic dose. Engraftment occurred in most patients at median of 24.5 days, taking into consideration that chimerism studies were obtain only weekly. Toxicities were similar to those reported in other studies in children. Additional studies are needed to determine the therapeutic efficacy of this single daily-dose approach in children undergoing SCT.

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