Cancer Chemother Pharmacol (2006) 57: 191–198
DOI 10.1007/s00280-005-0029-0

ORIGINAL ARTICLE

L. Nguyen · F. Leger · S. Lennon · C. Puozzo

Intravenous busulfan in adults prior to haematopoietic stem cell transplantation: a population pharmacokinetic study

Received: 21 December 2004 / Accepted: 18 March 2005 / Published online: 25 August 2005

Abstract An IV form of busulfan (IV Bu) has recently become available for high dose conditioning regimen before haematopoietic stem cell transplantation (HSCT). This IV form is expected to reduce the high pharmacokinetic variability exhibited with oral busulfan and as a result, to better target the plasma area under the curve (AUC). Pharmacokinetics (PK) of IV Bu was investigated on 127 adult patients (333 PK administrations) who received 0.8 mg · kg^{-1} of Bu as a 2-h infusion every 6 h over 4 days, followed by cyclophosphamide (60 mg·kg^{-1} day^{-1}×2). A retrospective population PK analysis was carried out to search for important predictive factors of IV Bu PK and to develop a limited sampling strategy (LSS) through Bayesian methodology. The analysis was conducted using the Non Linear Mixed Effect methodology and included a validation process on an independent data set. Adjusted Ideal Body Weight (AIBW) and Body Surface Area (BSA) were the best covariates to explain the inter-patient variability. The final inter-patient variability (CV = 16%) in IV Bu clearance (Cl_{tot}) was estimated close to the intra-patient variability (CV = 13%). There was neither age-dependency nor gender effect. IV Bu Cl_{tot} was not affected by elevated hepatic enzymes or by co-administration of either fluconazole or acetaminophen, and was not altered in heavily pre-treated or pre-transplanted patients. Normalised Cl_{tot} based on either AIBW or BSA was comparable between normal and obese patients (BMI = 18–26.9 kg·m^{-2}, > 26.9 kg·m^{-2}, respectively) whereas significant differences existed when based on either actual (ABW) or ideal body weight (IBW). As a consequence, no dose adjustment is required in obese patients when using a AIBW- or BSA-based dose calculation. A fixed dose of 0.80 mg·kg^{-1} of AIBW or 29 mg·m^{-2} of BSA yielded an average AUC of 1,200 μM·min, with 80% of patients within the "therapeutic" AUC range of 900–1,500 μM·min. Alternatively, 0.80 mg·kg^{-1} based on either ABW or IBW for normal patients and on AIBW for obese patients would achieve the same performance. A limited sampling strategy based on a Bayesian methodology was developed and validated on an independent dataset: AUCs obtained from one to two samplings were demonstrated to be reliably estimated.

Keywords Busulfan · Haematopoietic · Stem cell transplantation · Pharmacokinetic study

L. Nguyen (✉) · F. Leger · C. Puozzo
Institut de Recherche Pierre Fabre, Oncology Pharmacokinetics Department, 2 rue Christian d'Espic, 81106 Castres Cedex, France
E-mail: laurent.nguyen@pierre-fabre.com

S. Lennon
ESP Pharma, Edison, New Jersey, USA

Introduction

Busulfan is an alkylating agent commonly used in preparative regimens prior to haematopoietic stem cell transplantation (HSCT) in treatment of various malignancies and inherited disorders. For several decades, only an oral form of busulfan (oral Bu) has been available. Oral Bu high-dose in combination with cyclophosphamide (Cy) has become a widely used chemotherapy-based conditioning regimen prior to both allogeneic and autologous bone marrow transplantations (BMT). A standard oral Bu schedule is 1 mg/kg administered every 6 h during 4 days. As most of cytotoxic drugs, oral Bu exhibits a narrow therapeutic index. Several investigators associated the serious side effects of Bu-based therapy with systemic exposure. High areas under plasma concentrations-versus-time curve (AUC) are known to increase the risk of severe related-regimen toxicities including hepatic veno-occlusive disease (VOD) [6, 8, 14, 15, 33]. Conversely, low Bu AUCs are correlated with an increased risk of graft rejection and leukaemic relapse. Several reports [2, 8, 14, 32] indicate that a Bu AUC of 900–1,500 μM·min in adult patients receiving Bu q6h-based regimen prevents therapy failure without being associated with unacceptable severe toxicities or risk of developing VOD. PK of oral Bu were

extensively studied in both adults and children. Busulfan (Bu) is mainly eliminated through the liver [12, 13, 16] where it is converted into inactive metabolites by a glutathione-reductase-dependent mechanism involving glutathion-S-transferase enzymes [7, 27]. Renal elimination of Bu is limited; a mere 2% of the unchanged drug is excreted in urine [9, 20]. The magnitude of inter-patient variation associated with oral Bu apparent clearance (Cl/F) was estimated as high as 10-fold and more [15, 18, 20]. Patients' characteristics and treatment co-factors were examined to better understand this huge variability. Body size parameters such as Actual Body Weight (ABW), Body Surface Area (BSA), Ideal Body Weight (IBW), and Adjusted Ideal Body Weight (AIBW) have been correlated with Bu Cl/F [11, 30]. Age, obesity, diseases' specific variations, hepatic dysfunctions and drug–drug interactions might have contributed to the variability of oral Bu PK. Even when taking these factors into account, a PK-guided dose adjustment is still recommended to control the variability of oral Bu AUC. The unpredictable bioavailability of oral Bu [4, 22] prompted the development of an intravenous form of busulfan. This IV form of Bu in combination with Cy has been investigated during several clinical trials as pre-transplantation conditioning therapy in patients undergoing allogeneic and autologous HSCT for haematological cancers. During these studies, IV Bu doses were administered at a fixed dosing of 0.8 $mg{\cdot}kg^{-1}$ *q.i.d.* over 4 days. A 2-h infusion was selected in order to mimic the PK profile of oral Bu. PK data were collected from all the patients at several administrations. A population pharmacokinetic analysis using the Non Linear Mixed Effect methodology was performed from the pooled data. This retrospective analysis was aimed mainly at assessing both inter-patient and inter-dose variabilities, and defining dosing guidelines by searching relevant covariates that influence the Bu exposure. The other objective was to develop and then to validate a Limited Sampling Strategy (LSS) that will enable to determine the Bu AUC based on a very small number of plasma samples per patient.

Material and methods

Patients and dosing schedule

Data were obtained from 127 patients, including 103 patients from two phase II pivotal studies performed during the clinical development of IV Bu in USA between June 1996 and November 1997. Sixty-one and 42 patients were prepared for allogeneic and autologous transplants, respectively. Allogeneic patients were reported in a previous publication by Andersson et al. [1]. Autologous patients participated to a similar study. The remaining 24 patients were enrolled in three studies subsequent to the two-phase II pivotal trials. In all the trials, IV Bu was first administered over 4 days and followed by a 2-day therapy of cyclophosphamide at 60 $mg{\cdot}kg^{-1}$ daily. Transplantation was completed after one-day rest. The busulfan injection consisted of Bu (6 $mg{\cdot}ml^{-1}$) dissolved in dimethylacetamide (DMA, 33%, v/v) and polyethylene glycol-400 (PEG400, 67% v/v). The IV Bu dose was diluted in normal saline or 5% dextrose to approximately 0.5 mg/ml and infused via a controlled-rate infusion pump through a central catheter. Bu was administered intravenously at 0.8 $mg{\cdot}kg^{-1}$ over 2 h every 6 h for 16 doses. Sixty-eight percent of patients were dosed based on Ideal Body Weight (IBW), 23% on Actual Body Weight (ABW) and 9% on Adjusted Ideal Body Weight (AIBW). The following formulae were used to calculate IBW and AIBW [11]:

- Ideal body weight (IBW) :

$$\text{IBW (kg; men)} = 50 + 0.91 \times (\text{height} - 152)$$
$$\text{IBW (kg; women)} = 45 + 0.91 \times (\text{height} - 152)$$

with height expressed in cm.

- Adjusted ideal body weight (AIBW):

$$\text{AIBW (kg)} = \text{IBW} + 0.25 \times (\text{ABW} - \text{IBW})$$

with IBW and ABW expressed in kg.

No dose adjustment was made during the whole Bu therapy and phenytoin was administered as seizure prophylaxis.

Sampling and Bu determination

Plasma samples were collected following the first (day 1) and the ninth (day 2) doses at pre-infusion, 0.25, 0.5, 0.75 h after the start of the infusion, 5 min before the end of infusion and 0.25, 0.5, 1.0, 2.0, 3.0, and 4 h after the end of infusion. For dose 13 (day 3), samples were collected at pre-dose (before the start of infusion) and just before the end of infusion. Most samples were drawn from a peripheral IV line. All Bu concentrations were determined at a central laboratory using a validated Gas Chromatography method with Mass Selective detection (GC-MS) [34]. The limit of detection of the assay method was 62.5 $ng{\cdot}ml^{-1}$ and the within-run and between-run coefficients of variation were always below 10%.

Population pharmacokinetic model development

The population model development was carried out by Non linear Mixed Effect modelling, using the First Order With Conditional Estimation (FOCE) method within the NONMEM program (version 5.0) [3]. A covariate-free PK model (basic model) was first developed to best describe the concentration-time profiles of IV Bu. A one or a two-compartment pharmacokinetic model with a first order elimination from the central compartment was assessed. Inter-individual as well as inter-occasion variabilities (variability between doses 1, 9 and 13) were introduced into the model as exponential error models. Residual variability was described using an additive error model.

Current dosing practice involves a calculated Bu dosing based on different body-size parameters such as Actual Body Weight (ABW), Ideal Body Weight (IBW), Adjusted Ideal Body Weight (AIBW) and Body Surface Area (BSA). As a consequence, the respective influence of each of the body size parameters on IV Bu PK was evaluated using univariate NONMEM analyses. Direct linear relationships between body size parameter and Cl_{tot} were modelled [e.g. $Cl_{tot} = \theta_1.BSA$]. Each influence on Cl_{tot} was assessed from the improvement of fit between basic (covariate-free model) and tested model (with only one parameter) and its ability to explain the inter-patient variability. NONMEM calculates the Objective Function Value (OFV), which is a "goodness of fit" statistics. Any drop in OFV by more than 6.63 and 10.83 denotes an improved fit at $p < 0.01$ and $p < 0.001$, respectively.

The best "body size index model" was then selected and used to screen the influence of the other covariates (i.e. age, gender, biochemistry variables, prior chemotherapy, concomitant drugs, prior transplant and type of graft). They were alternatively included into the selected "body size index model" and those significantly improving the fit ($p < 0.01$) were subsequently entered into the model. Finally, the model was re-assessed using a backward elimination procedure. The modelling process was also guided by graphical approaches, plotting empirical Bayes estimates of PK parameters versus each covariate.

Impact of obesity on IV Bu clearance

Differences in normalised Cl_{tot} (from each parameter: ABW, IBW, AIBW or BSA) among categories of weights were evaluated. According to Body Mass Index (BMI), four weight categories were defined: underweight (BMI < 18 $kg{\cdot}m^{-2}$), normal (BMI 18–26.9 $kg{\cdot}m^{-2}$), obese (BMI 27–35 $kg{\cdot}m^{-2}$) and severely obese (BMI > 35 $kg{\cdot}m^{-2}$). BMI was calculated as Weight (kg)/Height^2(m^2) [11].

A first analysis was based on group comparisons using either one-way analyses of variance (ANOVA) or the equivalent non-parametric tests (Kruskall Wallis test). Then, a NONMEM modelling approach was used in order to confirm the first analysis. The impact of obese and severely obese covariates was tested on each body size index model: a decrease in OFV higher than 6.63 ($p < 0.01$) confirmed a significant improvement of fit. An example of model equation was: $Cl_{tot} = \theta_1.ABW.(1 + \theta_2$ Obese) $(1 + \theta_3$ Severely Obese) where obese and severely obese covariates were coded as 1 for obese or severely obese patients, otherwise zero.

Final model qualification and limited sampling strategy development

Final model equations and limited sampling strategies were assessed from a dataset never used for model development. This new validation dataset consisted of 24 patients (34 PK administrations: 12 at dose 1 and 22 at dose 9) enrolled in the three studies mentioned above. The final model was re-fitted on this validation dataset, and the resulting model parameters were compared to the initial ones from the training set ($n = 103$ patients); the model was qualified if there was no significant differences (Wald's test) between both sets of model parameters.

Once the model qualified, several schedules of LSS were generated (i.e. one to three sampling per dose and per patient). Empirical Bayes estimates of AUC_{inf} (dose 1) and AUC_{ss} (dose 9) from sparse sampling data were compared to the actual ones from full sampling data. Of note, the AUCs were calculated from the final population PK model developed from the training dataset. Mean prediction error as a measure of bias and Root Mean Square Error (RMSE) as a measure of precision were used to compare the various limited sampling designs. The best design required no significant bias to zero and the best precision (lowest RMSE value).

Results

Data

Concentrations data versus sampling time are presented on Fig. 1. The analysed dataset consisted of 2,207 concentrations from 103 patients and 299 administrations (PK data were missing from three and seven patients at doses 9 and 13, respectively). All patients were treated for haematological malignancies: 44 (43%) had lymphoma, 33 (32%) had acute leukaemia, 17 (16%) had chronic myelogenous leukaemia and 9 (9%) had a myelodysplasic syndrome. Body size parameters as well as the other demographic and biochemical characteristics recorded just before the Bu/Cy conditioning regimen are summarised in Table 1. Chemotherapy before Bu administration was received by 97 patients, 21 (20%) had received at least four prior chemotherapy courses (from

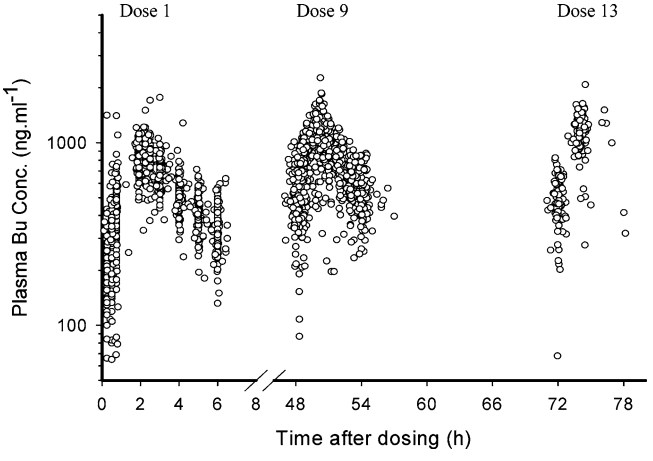

Fig. 1 Plasma IV Bu concentration versus time plot

Table 1 Patient characteristics

	Mean ± SD	Range
Demographic		
ABW (kg)	79.5 ± 18.7	41–125
AIBW (kg)	69.3 ± 11.2	47.2–95.1
IBW (kg)	65.8 ± 10.8	41.4–91.9
BSA (m^2)	1.94 ± 0.259	1.40–2.50
BMI (kg m^{-2})	26.9 ± 5.83	15.3–46.9
Age (years)	39.2 ± 11.4	19.0–64.0
Gender (M/F)	60/43	
Biochemistry		
Serum creatinine (mg dl^{-1})	0.848 ± 0.225	0.30–1.50
Clcr* (ml min^{-1})	126.6 ± 46.2	51.4–363.4
Serum albumin (g l^{-1})	3.49 ± 0.491	2.40–4.70
Total bilirubin (mg dl^{-1})	0.512 ± 0.240	0.10–1.70
Alcaline phosphatase (IU l^{-1})	98.0 ± 54.9	37.0–333
Lactate deshydrogenase (IU l^{-1})	460.6 ± 262.2	111–1743
Serum glutamyl pyruvate transaminase (IU l^{-1})	42.4 ± 38.7	8.0–215

* Creatinine clearance calculated according to the Cockroft and Gault formulae

four to eight courses), 36 (35%) had prior radiotherapy (from one to five courses), and 11 (11%) had prior transplants. When sufficient occurrences were available (at least 10% of patients), the impact of concomitant drugs on IV Bu Cl_{tot} was evaluated. The following associated drugs were tested: acetaminophen (18% of patients), fluconazole (13%) and anti-emetics 5-HT3 antagonists, mainly ondansetron (72%).

Population model development

A one-compartment model with first order elimination was suitable to describe concentrations versus time profiles. Concentrations below the limit of quantification were observed 15 min after the start of infusion (on the first dose) in about one third of patients. This was explained by the infusion of the hold-up volume not prefilled with the IV Bu solution. This delayed Bu infusion was modelled by introducing a lag compartment into the model, which resulted in high improvement of fit (drop in OFV > 1,000).

Body size parameters (BSA and ABW) were similarly and highly correlated with the Bu volume of distribution (Vc). The best fit was obtained when Vc was modelled as a power function of ABW: $Vc = \theta_2.ABW^{\theta 1}$, where θ_1 and θ_2 represent the slope and the intercept of the equation, respectively. The intercept was not different from 1 and the slope was 0.87. Consequently, the equation was simplified as $Vc = ABW^{\theta 1}$. This last model yielded a reduction for inter-individual variability in Vc from 24% (covariate-free model) to 13% (power function model).

Influence of body size parameters was further investigated on Cl_{tot}. Body size models for Cl_{tot} are reported in Table 2. There were no large differences between the body size index Cl_{tot} models, and the inter-individual variability ranged from 16.2% to 19.6% depending on the model. Nevertheless, the best improvement of fit (the highest drop in OFV) and the minimal inter-individual variability were obtained with the BSA-based model (see Table 2). The inter-individual variability in Cl_{tot} decreased from 22% (covariate-free model) to 16% (BSA model). BSA model parameters are reported in Table 3. All the other covariates were separately inserted into the BSA-based model; however there was no further improvement of fit (decrease in OFV < 6.63). Patient's biochemistry characteristics, gender, age, prior transplant, previous chemotherapies and/or radiotherapy showed no significant correlation with Bu Cl_{tot}. Concomitant drugs (fluconazole, acetaminophen and the 5HT-3 antagonists) had no influence.

Table 2 Body-size models

Models	Equation	OFV°	Inter-individual variability in IV Bu Cl_{tot}
BSA model	$Cl_{tot}(l.h^{-1}) = 5.96.BSA$	22,614	16.2%
ABW model	$Cl_{tot}(l.h^{-1}) = 0.148.ABW$	22,637	19.6%
IBW model	$Cl_{tot}(l.h^{-1}) = 0.176.IBW$	22,656	19.3%
AIBW model	$Cl_{tot}(l.h^{-1}) = 0.167.AIBW$	22,632	16.7%

°*OFV* objective function value produced by NONMEM

Final model qualification and limited sampling strategy

Patients' characteristics were similar between the validation ($n = 24$ patients) and the training datasets ($n = 103$ patients). Age, BSA and ABW in the validation dataset ranged from 14.3 years to 64 years, 1.41 m^2 to 2.44 m^2, and 43.3 kg to 115 kg, respectively.

The stability of the final model was demonstrated through the similarity of model parameters obtained from either the training or the validation dataset (see Table 3).

Bias and precisions of AUC calculations of each tested LSS are reported in Table 4. The best result was obtained with a two-sampling strategy (15 min and 4 h after end of infusion) (see Fig. 2); i.e. no significant bias and better precision (lower RMSE value). Using this

Table 3 Final parameters of BSA-based model

Dataset		Training set ($n=103$)		Validation set ($n=24$)	
		Parameter	SE (%)	Parameter	SE (%)
Cl ($l.h^{-1}$)	θ 1 .BSA	5.96	2	5.91	5
Vc (l)	ABW θ2	0.870	1	0.902	1
Lag time—K12 (h^{-1})	θ 3	70.2	35	NE	NE
Inter-individual variability (IIV)					
	IIV_Cl	16%	23	23%	34
	IIV_Vc	13%	24	15%	30
	IIV_ K_{12}	300%	22	NE	NE
Inter-dose variability					
	IDV_Cl	13%	19	10%	38
Correlation ClvsVc		0.53	33	0.61	53
Residual ($ng{\cdot}ml^{-1}$)		79.2	10	69.5	12

NE not estimated
SE standard error (%)

LSS, the absolute prediction error was < 10% in 30 out of the 34 evaluated administrations whereas it was < 14% in the four remaining administrations. A one-sampling strategy (early sample collected 30 min to 1 h after the end of infusion) also provided acceptable results without any significant bias and with a good precision (RMSE < 10%). When using only one sample but later-collected (from 4 h to 6 h after dosing), the bias was significantly different from zero and the precision was poor in some patients (absolute prediction error > 20%).

Impact of obesity on IV Bu clearance

This analysis was conducted combining the training and the validation datasets ($n=127$ patients). There were six underweight (BMI < 18 $kg{\cdot}m^{-2}$), 71 normal (BMI: 18–26.9 $kg{\cdot}m^{-2}$), 39 obese (BMI: 27–35 $kg{\cdot}m^{-2}$) and 11 severely obese (BMI > 35 $kg{\cdot}m^{-2}$) patients.

Comparisons of normalised Cl_{tot} among categories of weight are reported in Table 5. No significant difference among weight groups was illustrated when IV Bu Cl_{tot} was normalised using either BSA or AIBW, whereas ABW and IBW-based normalisation showed statistically significant differences ($p<0.01$) between normal and obese, and normal and severely obese patients. Cl_{tot}/ABW was 11% lower in obese and 28% lower in severely obese patients than in normal patients. Cl_{tot}/IBW was 13% higher in obese and 24% higher in severely obese patients than in normal patients. These results were confirmed with the population modelling approach. When included into the BSA or AIBW body size models, the obese and severely obese covariates did not improve the fit, while these covariates showed a significant improvement of fit ($p<0.01$) when included into the ABW or the IBW model.

Discussion

This population PK study on IV Bu was performed on a representative adult population treated with standard BuCy regimen prior to haematopoietic stem cell transplantation. Previous studies were carried out in an attempt to explain the large variability of oral Bu that is known to be extensively metabolised by the liver [11, 12, 15] through the glutathione (GSH) conjugation pathway. Literature indicates that alteration of the liver function could affect the elimination of oral Bu. From a population PK analysis on 74 patients, Sandstrom et al. [28] found that the clearance of oral Bu was reduced in patients with elevated alkaline phosphatases (ALP). Another study by Hassan M et al. [18] showed high oral Bu concentrations in a patient with elevated liver transaminases. In our study, neither ALP above the upper normal limits nor elevated SGPT affected the total body clearance of IV Bu.

Table 4 Prediction of AUC_{inf} (first dose) and AUC_{ss} (dose 9) using limited sampling strategies

Limited sampling strategy	Sampling time after dosing°					
	2 h 15 min and 6 h (%)	2 h 30 min (%)	3 h (%)	4 h (%)	5 h (%)	6 h (%)
Bias mean prediction error	−0.32	−0.90	−0.94	+3.7*	+7.0*	+10.4*
SE ($CI_{95\%}$)	[−2.3 to 1.7]	[−3.3 to 1.5]	[−3.3 to 1.4]	[0.7 to 6.6]	[3.2 to 11]	[5.3 to 15]
RMSE	5.9	7.1	6.9	9.4	13	18

* Significant bias to zero with student t-test° Sampling strategies included a trough concentration at dose 9

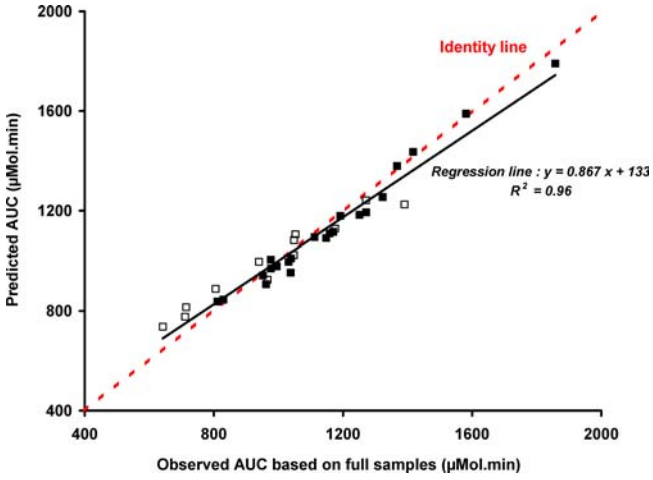

Fig. 2 Predicted AUC based on two-samples (2.5 h and 6 h) versus observed AUC based on the full samples set. *Empty squares* are doses 1 and *solid squares* are dose 9. *Dotted line* is the identity line and *solid line* is the regression line

Elevated serum creatinine or low creatinine clearance was not correlated with IV Bu Cl_{tot}, which was expected since oral Bu renal elimination is known to be very limited [9, 20].

Concerns on drug interactions with oral Bu were reported in literature data [25]. To prevent seizure either phenytoin or benzodiazepine is frequently associated with Bu-based therapy. Phenytoin is known to be a strong hepatic enzymes inducer [23] although no impact on glutathione-S-transferases has been reported. Therefore, the real impact of phenytoin on Bu metabolism remains a controversial issue. Some authors [19, 28] suggested that patients with phenytoin presented a significantly higher oral Bu clearance than patients with benzodiazepines, and that the impact of phenytoin was increasing over the treatment period: Cl/F calculated at the last oral Bu administration was increased by 20%. On the other hand, Embree et al. [10] did not observe any induction of oral Bu Cl/F when comparing phenytoin with diazepam groups. In our study, all patients but one received phenytoin as seizure prophylaxis and therefore comparative analysis was impossible. Nevertheless, should an induction have occurred during the treatment period IV Bu clearance would have increased from dose 1 to dose 13. Conversely, the average IV Bu clearance tended to be slightly lower at dose 9 ($Cl = 11.2 \pm 2.74$ l·h^{-1}) and dose 13 ($Cl = 11.1 \pm 2.64$ l·h^{-1}) than at dose 1 ($Cl = 12.6 \pm 3.04$ l·h^{-1}). Therefore, an induction is unlikely when phenytoin is concomitantly administered with IV Bu for 4 days.

Anti-fungal imidazole prophylaxis is commonly used in BMT patients. Although the involved mechanism is unclear, some authors [5, 21, 26] suggested an impact of imidazole compounds on oral Bu metabolism. In our study 13 patients received IV Bu with fluconazole. No impact was demonstrated on IV Bu clearance, confirming the results from oral Bu and fluconazole obtained by Buggia et al. [5]. In her study, it was observed that itraconazole significantly increased the plasma exposure of oral Bu. In our study only one patient received itraconazole during the whole IV Bu therapy. Although no formal conclusion could be drawn with one case data it is worth noting that this patient presented a constant IV Bu AUC of 1,111, 1,185 and 1,154 µM·min at doses 1, 9 and 13, respectively. These AUC values were within the therapeutic range of 900–1,500 µM·min and were comparable to those from other patients. It has also been suggested that metronidazole decreased oral Bu Cl/F [26]. In our study, the only patient receiving metronidazole the first 2 days of the IV Bu therapy had a standard AUC_{inf} at dose 1 of 1,082 µM·min.

Although there is no mention in literature of any case of interaction with oral Bu, acetaminophen could be suspected for its ability to decrease the glutathione levels in blood and tissues [30]. In our study, 19 patients received acetaminophen with no modification of IV Bu clearance.

Prior transplant and chemotherapies are risk factors of developing VOD. Since Bu exposure is a determinant of VOD, the impact of these covariates was investigated. However, the IV Bu clearance was not altered in patients who received 4–8 courses of prior chemotherapies ($n = 21$) and/or underwent prior transplant ($n = 11$).

Among the tested covariates, only body size parameters showed significant correlations with IV Bu Cl_{tot}. BSA and AIBW were the best determinants to explain the inter-individual variability, followed with ABW and IBW. The impact of obesity was investigated on each of the body size index models (i.e. BSA, AIBW, ABW and IBW models). Obesity had no impact on BSA and AIBW models whereas a significant influence was demonstrated on ABW and IBW models. Cl_{tot}/ABW was respectively 11 and 28% lower in obese and severely obese patients than in normal ones (see Table 5). Similar

Table 5 Busulfan Cl_{tot} in underweight (BMI < 18 kg·m^{-2}), normal (BMI = 18–26.9 kg·m^{-2}), obese (BMI = 27–35 kg·m^{-2}) and severely obese patients (BMI > 35 kg·m^{-2})

	Underweight	Normal	Obese	Severely obese
n	6	71	39	11
Cl_{tot}/BSA (ml·min^{-1}·m^{-2})	94.1 ± 22.8	98.9 ± 18.5	100.3 ± 21.5	89.4 ± 17.4
Cl_{tot}/AIBW (ml·min^{-1}·kg^{-1})	2.48 ± 0.633	2.72 ± 0.550	2.87 ± 0.617	2.82 ± 0.450
Cl_{tot}/ABW (ml·min^{-1}·kg^{-1})	2.95 ± 0.667	2.63 ± 0.533	2.33 ± 0.517*	1.88 ± 0.383*
Cl_{tot}/IBW (ml·min^{-1}·kg^{-1})	2.35 ± 0.633	2.75 ± 0.567	3.10 ± 0.667*	3.40 ± 0.467*

* Significant difference ($p < 0.05$) compared with normal patients (Kruskall Wallis test)

conclusions were reached by Gibbs et al. [10] who studied oral Bu PK from a database of 279 adults: respectively 12% and 21% lower in obese and severely obese than in normal patients when oral Bu clearance was normalized to ABW, and no differences when oral Bu clearance was normalized to BSA or AIBW. From the above, should normal and obese patients be administered the same dose, the Bu dose (oral and IV) calculation must be done on either BSA or AIBW. In other words no dose adjustments are required in obese patients when using BSA or AIBW method of dose calculations. However, dose adjustment is needed when dose calculation is based on ABW or IBW. Consequently, BSA or AIBW calculated dosing should be favoured for easy practice. From the developed models (see Table 2), a 29 mg·m^{-2} of BSA or a 0.80 mg·kg^{-1} of AIBW IV Bu dose would achieve a plasma exposure of about 1,200 μM·min, the median value of the therapeutic window [900–1,500 μM·min]. Nevertheless, many centres are still using ABW in normal patients, unless there are some concerns about obesity. In such a case, to ensure that all patients receive an equivalent dose level (0.80 mg·kg^{-1}), the dosing calculation should be based on ABW and on AIBW in normal and obese patients (BMI > 27 kg·m^{-2}), respectively.

From studied data, IV Bu clearance normalized to ABW or BSA appeared to be not significantly different between normal and underweight patients. These results suggested that ABW- or BSA-dosing calculation should be recommended in underweight patients without any further dose adjustment. However, due to the small number of patients in this group (BMI from 15.3 kg·m^{-2} to 17.7 kg·m^{-2}), further analyses are needed on a larger sample size to reach a clear conclusion.

The AUC targeting performance to achieve the therapeutic window was simulated for several methods of dosing calculation (see Fig. 3). About 80% of the 127 patients would be within the targeted AUC range following IV Bu administration of either 29 mg·m^{-2} of BSA or 0.80 mg·kg^{-1} of AIBW. The same performance would be obtained with 0.80 mg·kg^{-1} of ABW or IBW in normal patients, and with 0.80 mg·kg^{-1} of AIBW in obese patients (BMI > 27 kg·m^{-2}). 0.80 mg·kg^{-1} of ABW or IBW in all patients resulted in lower performance. Of clinical importance, the mathematical simulation suggested that ABW-based dosing in obese patients would result in plasma over-exposure (44% of AUCs from 1,500 μM·min to 2,173 μM·min) likely to increase the risk of regimen-related toxicities, whilst the IBW-based dosing would result in an under-exposure (17% of AUCs from 536 μM·min to 900 μM·min) likely to enhance the risk of graft failure.

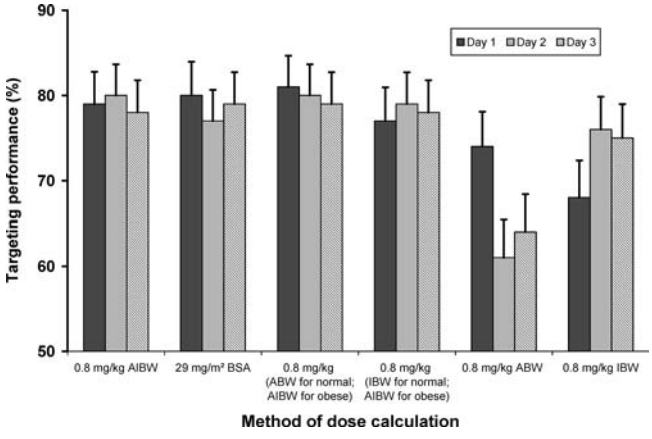

Fig. 3 Percent of patients within the targeted AUC from [900 to 1500 μM·min] using different methods of dose calculation

The population analysis estimated an inter-dose variability of about 13% in the Cl_{tot} of IV Bu, which was in line with earlier reports on IV Bu using either different formulations [17, 29] or the same one [1, 24]. As a consequence, the AUC is expected to be reproducible all along the treatment. The consistency of AUC over administrations is illustrated by a constant targeting performance (80% of patients within the target AUC) throughout the PK control of doses 1, 9 and 13 (see Fig. 3).

A limited sampling strategy using Bayesian methodology was developed. A reliable AUC estimated from two plasma concentrations (15 min after the end of infusion, and just before the next administration) is validated. Acceptable estimation of AUC remains possible when using only one plasma concentration within the first hour post-infusion. A later plasma concentration (> 3 h post-dosing) is less informative.

In conclusion, this population PK analysis demonstrated that body size parameters were the only significant determinants in the pharmacokinetics of IV Bu. BSA and AIBW best explained the inter-individual variability. Dosing based on the above mentioned body size parameters does not necessitate adjustment in obese and severely obese patients. This paper provides limited sampling strategies based on one or two plasma concentrations per patient and per administration for further investigations on IV Bu pharmacokinetics and pharmacokinetic/pharmacodynamic relationships.

References

1. Andersson BS, Kashyap A, Gian V et al (2002) Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: a phase II study. Biol Blood Marrow Transplant 8:145–154
2. Andersson BS, Thall PF, Madden T et al (2002) Busulfan systemic exposure relative to regimen-related toxicity and acute graft-versus-host disease: defining a therapeutic window for IV BuCy2 in chronic myelogenous leukaemia. Biol Blood Marrow Transplant 8:477–485
3. Beal SL, Sheiner LB (1998) NONMEM user's guide. University of California, San Francisco
4. Bostrom B, Enockson K, Johnson A et al (2003) Plasma pharmacokinetics of high-dose oral busulfan in children and adults undergoing bone marrow transplantation. Pediatr Transplant 7(suppl 3):12–18
5. Buggia I, Zecca M, Alessandrino EP et al (1996) Itraconazole can increase systemic exposure to busulfan in patients given bone marrow transplantation. Anticancer Res 16:2083–2088

6. Copelan EA, Bechtel TP, Avalos BR et al (2001) Conditioning regimens: busulfan levels are influenced by prior treatment and are associated with hepatic veno-occlusive disease and early mortality but not with delayed complications following marrow transplantation. Bone Marrow Transplant 27:1121–1124
7. Czerwinsky M, Gibbs JP, Slattery JT (1996) Busulfan conjugation by glutathione *S*-transferases α, μ, and π. Drug Metab Dispos 24:1015–1019
8. Dix SP, Wingard JR, Mullins RE et al (1996) Association of busulfan area under the curve with veno-occlusive disease following BMT. Bone Marrow Transplant 17:225–230
9. Ehrsson H, Hassan M, Ehrnebo M, Beran M (1983) Busulan kinetics. Clin Pharmacol Ther 34:86–89
10. Embree L, Heggie JR, Knight G et al (1997) Effect of phenytoin on busulfan pharmacokinetics. Pharm Res 14, 11, suppl. S613–abstract#3455
11. Gibbs JP, Gooley T, Borneau B et al (1999) The impact of obesity and disease on busulfan oral clearance in adults. Blood 93(12):4436–4440
12. Gibbs JP, Liacouras CA, Baldassano RN, Slattery JT (1999) Up-regulation of glutathione *S*-transferase activity in enterocytes of young children. Drug Metab Dispos 27:1466–1469
13. Gibbs JP, Murray G, Risler L et al (1997) Age-dependent tetrahydrothiophenium ion formation in young children and adults receiving high-dose busulfan. Cancer Res 57:5509–5516
14. Grochow LB (1993) Busulfan disposition: the role of therapeutic monitoring in bone marrow transplantation induction regimens. Semin Oncol 20(4 suppl 4):18–25
15. Grochow LB, Jones RJ, Brundrett RB et al (1989) Pharmacokinetics of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. Cancer Chemother Pharmacol 25:55–61
16. Hassan M, Ljungman P, Bolme P et al (1994) Busulfan bioavailability. Blood 84:2144–2150
17. Hassan M, Nilsson C, Hassan Z et al (2002) A phase II trial of liposomal busulphan as an intravenous yeloablative agent prior to stem cell transplantation: 500 mg/m^2 as a optimal total dose for conditioning. Bone Marrow Transplant 30:833–841
18. Hassan M, Oberg G, Bekassi AN et al (1991) Pharmacokinetics of high-dose busulphan in relation to age and chronopharmacology. Cancer Chemother Pharmacol 28:130–134
19. Hassan M, Oberg G, Björkholm M et al (1993) Influence of prophylactic anticonvulsant therapy on high-dose busulphan kinetics. Cancer Chemother Pharmacol 33:181–186
20. Hassan M, Oberg G, Ehrsson H et al (1989) Pharmacokinetic and metabolic studies of high-dose busulphan in adults. Eur J Clin Pharmacol 36:525–530
21. Leather HL (2004) Mini-review: drug interactions in the hematopoietic stem cell transplant (HSCT) recipient: what every transplanter needs to know. Bone Marrow Transplant 33:137–152
22. Lindley C, Shea T, McCune J et al (2004) Intraindividual variability in busulfan pharmacokinetics in patients undergoing a bone marrow transplant: assessment of a test dose and first dose strategy. Anti-Cancer Drugs 15:453–459
23. Nation RL, Evan AM, Milne RW (1990) Pharmacokinetic drug interactions with phenytoin (part I). Clin Pharmacokinet 18(1):37–60
24. Nguyen L, Fuller D, Lennon S, Leger F, Puozzo C (2004) IV busulfan in pediatrics: a novel dosing to improve safety/efficacy for hematopoietic progenitor cell transplantation recipients. Bone Marrow Transplant 33(10):979–987
25. Nieto Y, Vaughan WP (2004) Pharmacokinetics of high-dose chemotherapy. Bone Marrow Transplant 33:259–269
26. Nilsson C, Aschan J, Hentschke P et al (2003) Conditioning regimens: the effect of metronidazole on busulfan pharmacokinetics in patients undergoing hematopoietic stem cell transplantation. Bone Marrow Transplant 31:429–435
27. Poonkushali B, Chandy M, Srivastava A et al (2000) Glutathione *S*-transferase activity influences busulfan pharmacokinetics in patients with beta thalassemia major undergoing bone marrow transplantation. Drug Metab Dispos 29:264–267
28. Sandström M, Karlsson MO, Ljungman P et al (2001) Population pharmacokinetic analysis resulting in a tool for dose individualization of busulphan in bone marrow transplantation recipients. Bone Marrow Transplant 28:657–664
29. Schuler U, Renner UD, Kroschinsky F et al (2001) Intravenous busulphan for conditioning before autologous or allogeneic human blood stem cell transplantation. Br J Haematol 114:944–950
30. Schuler U, Schroer S, Kühnle A et al (1994) Busulfan pharmacokinetics in bone marrow transplant patient: is drug monitoring warranted? Bone Marrow Transplant 14:759–765
31. Shulman HM, Hinterberg W (1992) Hepatic veno-occlusive disease—liver toxicity syndrome after bone marrow transplantation. Bone Marrow Transplant 10:197–214
32. Slattery JT, Clift RA, Buckner CD et al (1997) Marrow transplantation for chronic myeloid leukaemia: the influence of plasma busulfan levels on the outcome of transplantation. Blood 89:3055–3060
33. Slattery JT, Sanders JE, Buckner CD et al. (1995) Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. Bone Marrow Transplant 16:31–42
34. Vassal G, Ré M, Gouyette A (1988) Gas chromatographic-mass spectrometry assay for busulfan in biological fluids using deuterated internal standard. J Chromatogr 428:357