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Exposure equivalence between IV (0.8 mg/kg) and oral (1 mg/kg) busulfan in adult patients

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Abstract

Objective This study was conducted to retrospectively compare the area under the curve (AUC) and the total clearance of busulfan (Bu) following oral and intravenous (IV) administrations and to determine which intravenous dose generated equivalent exposure to that of the oral form that has been marketed for decades.

Methods Patient pharmacokinetics were assessed at dose 9 during a conditioning regimen for stem-cell transplantation and included data from 277 patients for oral Bu (71 from fixed-dose studies and 206 from studies with dose adjustment allowed) and 120 patients for IV Bu (fixed dose). AUCs were compared between patients with fixed dose of oral Bu ($n=71$, 1 mg/kg) and those of IV Bu ($n=120$, 0.8 mg/kg). Total clearances were calculated for all 277 patients with oral Bu and compared to those with IV Bu, with the ratio of IV-to-oral clearance representing the absolute bioavailability of the oral form.

Results Oral and IV populations differed on disease-type distribution but presented comparable demography parameters. IV Bu dosing was mostly based on the ideal body weight index while actual body weight or adjusted ideal body weight indexes were mostly used for oral. When normalised to comparable indexes, bioequivalent AUCs were achieved between oral and IV populations. Oral Bu bioavailability was about 80% when calculated from the ratio of IV-to-oral total clearances.

Conclusion This retrospective study carried out on a large set of data showed that similar plasma exposures were achieved with 1.0 mg/kg oral Bu or 0.8 mg/kg IV Bu.

Keywords Pharmacokinetics · Bioequivalence · Haematopoietic stem cell transplantation (HSCT) · Actual body weight (ABW) · Adjusted ideal body weight (AIBW)

Introduction

Busulfan (Bu) is an alkylating agent commonly used in myeloablative conditioning regimens before haematopoietic stem cell transplantation (HSCT) for treatment of various malignancies and inherited disorders. High doses of oral Bu in combination with cyclophosphamide (Cy) have become a widely used chemotherapy-based conditioning regimen prior to both allogeneic and autologous bone marrow transplantations (BMT). The usual total dose is 16 mg/kg administered as 1 mg/kg every 6 h for 4 days.

Pharmacokinetics (PK) of oral Bu has been extensively studied in both adults and children. Busulfan is mainly eliminated by the liver [1–3] where it is converted into inactive metabolites by a glutathione-reductase-dependent mechanism involving glutathion-S-transferase enzymes [4, 5]. Renal elimination of Bu is known to be minor; about 2% of the unchanged drug is excreted in the urine [6, 7]. The magnitude of inter-patient variation associated with oral Bu apparent clearance (CL/F) has been estimated at as high as 10-fold or more [7–9]. Patient characteristics and treatment co-factors have been studied for a better understanding of this high variability. Body-size descriptors such as actual body weight (ABW), body surface area (BSA), ideal body weight (IBW) or adjusted ideal body weight (AIBW) have been correlated with Bu CL/F [10,

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11]. Age, obesity, disease-specific variations, hepatic dysfunctions and drug-drug interactions have been suggested to contribute to the high variability associated with oral Bu PK.

As with most cytotoxic drugs, oral Bu exhibits a narrow therapeutic index. High values of areas under the plasma concentration-versus-time curve (AUC) have been related to an increased risk of severe regimen-related toxicities including hepatic veno-occlusive disease (VOD) [8, 12–15]. Conversely, low Bu AUCs have been correlated with an increased risk for graft rejection and leukaemia relapse. The most commonly accepted range of therapeutic AUC is 900–1,500 $\mu\text{M}\cdot\text{min}$ in adult patients receiving oral Bu four times a day for 4 days [13, 14, 16, 17].

A pharmacokinetically guided dose adjustment has been developed and is used in some clinical centres to circumvent the intra- and inter-patient variability in Bu exposure following oral Bu administration [11, 12, 17–19].

The unpredictable bioavailability of oral Bu associated with both high inter- and intra-patient variabilities [18, 19] prompted the development of an intravenous formulation. It appeared complex to develop due to the very poor aqueous solubility of Bu and its chemical instability when solubilised in pharmaceutical formulations. Several attempts to develop an IV formulation have been conducted [3, 20]; one formulation (Busilvex) has been approved in the U.S. and Europe and is indicated for the conditioning regimen prior to stem cell transplantation. This IV form of Bu, in combination with cyclophosphamide or melphalan, has been investigated in several clinical trials as a pre-transplantation conditioning therapy for patients undergoing allogeneic and autologous HSCT for haematological cancers [21–24]. During these studies, the PK of IV Bu was investigated with Bu being administered at 0.8 mg/kg as a 2-h infusion under the same schedule as the one used for the oral formulation (every 6 h over 4 days). The dose was infused over 2 h to achieve a similar peak to that achieved with oral Bu administration.

The equivalence of oral versus IV doses producing comparable exposures of Bu was explored during a phase I trial on a limited number of patients [20]. Therefore, further analyses based on both a larger number of datasets and obtained at steady state were needed to definitively address the issue of whether oral versus IV doses achieved similar exposures and thereby the Bu bioavailability value. The aim of this study was to retrospectively compare the area under the curve and the total clearance of Bu between oral and IV administrations, in order to demonstrate equivalent exposures between a 1 mg/kg oral Bu dose and a 0.8 mg/kg dose of IV Bu formulation (Busilvex) from large datasets collected at the same steady-state occasion.

Methods

Patients

Patients receiving IV Bu were part of five phase II clinical trials conducted between 1995 and 1998 during the clinical U.S. development program of IV Bu.

Individual PK data from the patients receiving oral Bu were collected between 1992 and 1996 as part of routine clinical Bu monitoring at the Fred Hutchison Cancer Research Center (FHCRC; Seattle, WA, USA).

The FHCRC database was screened for adult patients who received oral Bu as a part of the BuCy2 preparative conditioning regimen prior to haematopoietic progenitor cell transplantation, which represented a total of 277 patients.

Patient characteristics are summarised in Table 1.

Based on body mass index (BMI), four weight categories were defined: underweight (BMI < 18 kg/m²), normal (BMI 18–26.9 kg/m²), obese (BMI 27–35 kg/m²) and

Table 1 Summary of patient characteristics

Characteristics	Oral data (fixed-dose patients)	Oral data (all patients)	IV data
Number of patients	71	277	120
Age (years)	42±10 (21–61)	44±10 (18–66)	39±12 (14–64)
Actual body weight (kg)	77±12 (50–103)	77±16 (47–131)	79±19 (41–122)
Male/female (%)	66/34	52/48	56/44
Body mass index (kg/m ²)	26±3.9 (17–34)	26±4.9 (17–44)	27±5.8 (15–46)
Weight category			
Underweight	3 (4)	3 (1)	6 (5)
Normal	37 (52)	171 (62)	61 (51)
Obese	31 (44)	87 (31)	42 (35)
Severely obese	0	16 (6)	11 (9)
Disease			
Acute leukaemia	8 (11)	38 (14)	34 (28)
Chronic myelogenous leukaemia	51 (72)	156 (56)	23 (19)
Myelodysplastic syndrome	3 (4)	51 (18)	11 (9)
Myeloproliferative disorders	1 (2)	22 (8)	0
Lymphoma	2 (3)	3 (1)	18 (15)
Hodgkin's disease	0	0	34 (28)
Solid tumor	6 (8)	7 (3)	0

Unless otherwise stated, data are presented as mean ± SD with range *in brackets* or as number of patients with frequency (%) *in brackets*

severely obese (BMI > 35 kg/m²). BMI was calculated as weight (kg) divided by the square of height (m²) [10].

Procedure

Patients were conditioned for either allogeneic or autologous transplants. The same preparative conditioning regimen (BuCy2) for haematological malignancies was used for all patients. It consisted of a standard 16-dose regimen of either oral or IV Bu administered every 6 h for 4 days. This treatment was followed by a standard cyclophosphamide regimen (120 mg/kg). Phenytoin was used as a seizure prophylaxis treatment.

IV Bu was administered by intravenous infusion of 0.8 mg/kg over 2 h every 6 h for 16 doses. The Bu injection consisted of Bu (6 mg/ml) 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. The IV dose of Bu was calculated based on actual, ideal or adjusted ideal body weight, the choice depending on the participating institution's practice. No dose adjustment was performed during the whole Bu therapy.

The following formulae were used to calculate ideal body weight (IBW) and adjusted ideal body weight (AIBW) [10]:

$$\text{For men : IBW (kg)} = 50 + 0.91 \times (\text{height} - 152)$$

$$\text{For women : IBW (kg)} = 45 + 0.91 \times (\text{height} - 152)$$

with height expressed in centimetres.

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

with IBW and ABW expressed in kilograms.

For oral Bu, a standard Bu starting dose of 1.0 mg/kg was administered as Bu tablets. No other cytotoxic agents or irradiation (TBI) was administered immediately before or concomitantly with Bu. The oral dose of Bu was calculated based on either actual or adjusted IBW according to the institution's practice. Data from 277 patients were available, of which 71 patients were enrolled in fixed-dose BuCy2 protocols and the other 206 patients were enrolled in protocols that allowed Bu dose adjustment for AUC targeting.

Information about patients who might have vomited following oral Bu administrations was not available in the provided database. Therefore, although this adverse effect probably occurred in some patients, the analysis was carried out on the total number of patients.

Sampling and Bu determination

Blood samples for the IV-Bu patients were collected after the first (day 1) and the ninth (day 2) doses. Samples were drawn just before, 0.25, 0.5, 0.75, 2, 2.25, 2.5, 3, 4, 5 and 6 h after the start of infusion. All samples were collected from a peripheral IV line.

Plasma samples for the oral-Bu patients were obtained following administrations 1, 5, 9 and 13. Typically, samples were drawn just before and 1, 2, 3, 4 and 6 h after the administration of Bu.

For the purpose of the study, only steady-state data were considered because of the lower variability generally observed and more reliable AUC estimates obtained at steady state compared with dose 1. Since most oral and IV PK samples were collected after the ninth administration, oral and IV exposures were compared at this occasion. AUCs were calculated based on the full dosing interval, i.e. AUC_{0–6 h}. This represented a total of 120 patients for IV Bu and 277 patients for oral Bu.

Drug assay

The same analytical method was used for both oral and IV Bu concentration determination. All Bu concentrations were analysed at a central laboratory using a validated gas chromatography method with mass selective detection (GC-MSD) [25]. The limit of detection of the assay method was 62.5 ng/ml and the within-run and between-run coefficients of variation were always below 10%.

Pharmacokinetic analysis

Graphical comparison of individual AUCs at steady state was performed using box-plot representations, enabling the median, 25th, 75th percentiles as well as outlier values to be visualised.

IV Bu AUCs were calculated by non-compartmental analysis with the trapezoidal rule, using Kinetica Software version 4.1 (Innaphase, USA). Oral Bu AUCs were also calculated by non-compartmental analysis, as previously described [15].

Statistical analyses were performed using the SAS program (version 8.02, SAS Institute, Cary, NC, USA). Mean, median, standard deviation, and coefficient of variation minimum and maximum values were calculated for inter-route comparisons. The statistical tests for AUC comparison were performed using a natural-log scale, assuming a log normal distribution of AUC. Bartlett's statistic was used to test for homoscedasticity.

First, IV and oral mean AUCs were compared using a one-way analysis of variance (proc GLM) with a "route" effect. The alpha risk was set at 5%. The comparison was

performed on a reduced oral dataset (71 patients) only including patients who had not been dose-adjusted during the treatment with oral Bu (i.e. those on a fixed 1 mg/kg dose).

Then, the 90% confidence interval of the difference between mean values of log-transformed AUCs was computed as:

$$100 \times e^{\ln(\text{AUC}_{\text{oral}}) - \ln(\text{AUC}_{\text{iv}}) \pm t_{0.10, d11} \times \sqrt{(MSE \times (1/N_{\text{iv}} + 1/N_{\text{oral}}))}}$$

where $t_{0.10, d11}$ is the student t -test value calculated for 10% bilateral alpha risk with $d11$ being the degrees of freedom ($N_{\text{iv}} + N_{\text{oral}} - 2$); MSE is the mean square error of the ANOVA test and N_{iv} and N_{oral} are the sample sizes of the IV and oral groups, respectively.

As stated in the CPMP and FDA guidelines [12, 26], equivalence of exposure was concluded if the calculated 90% confidence interval was within the acceptance range (0.80–1.25).

The oral dataset provided by FHCRC contained 277 patients of which 206 patients were involved in protocols that allowed dose-adjustment (targeting protocols). Direct comparison of AUCs was therefore excluded since steady-state oral doses could differ from 1 mg/kg if dose adjustments were performed. However Bu PK is linear, at least in the usual range of therapeutic doses, enabling a dose-adjustment strategy. Thus, if exposures were dose-normalised (AUC/dose), full datasets could be used. The total clearance, which represents the opposite ratio (dose/AUC), was used to evaluate the bioavailability of oral Bu on full datasets ($F = \text{Cl}_{\text{iv}}/\text{Cl}_{\text{oral}}$). The major value of this parameter is that, in contrast to AUC, the clearance is not influenced by the body mass index used for the dosing.

Bioavailability was first evaluated on the complete dataset of patients ($n=277$) and then on the reduced dataset ($n=71$ patients included in fixed-dose protocols with oral Bu). Average absolute bioavailability (F) was calculated as the ratio between the mean total drug clearance following IV administration and the mean apparent drug clearance following oral administration. On the natural logarithm scale, the following formula was applied:

$$\begin{aligned} \text{Mean Ln}(F) &= \text{Mean Ln}(\text{Total IV Cl}) \\ &\quad - \text{Mean Ln}(\text{Apparent Oral Cl}) \end{aligned}$$

A one-way ANOVA procedure was performed in order to calculate the residual mean square error (MSE). Afterwards, the 95% confidence interval was calculated as follows:

$$\text{Cl}_{95\%} = 100 \times e^{\ln(\text{Cl}_{\text{oral}}) - \ln(\text{Cl}_{\text{iv}}) \pm t_{0.05, d11} \times \sqrt{(MSE \times (1/N_{\text{iv}} + 1/N_{\text{oral}}))}}$$

where $t_{0.05, d11}$ is the calculated student value for 5% bilateral alpha risk with $d11$ being the degrees of freedom

($N_{\text{iv}} + N_{\text{oral}} - 2$); MSE is the mean square error of the ANOVA test and N_{iv} and N_{oral} are the sample sizes of the IV and oral groups, respectively.

Results

Comparison of oral and IV datasets

Oral and IV groups of patients presented similar demographic characteristics (see Table 1). No significant differences in age, actual body weight, gender ratio or body mass index were detected through statistical comparisons of means (t -test) or proportions (chi-squared). Underweight and severely obese patients were slightly more represented in the IV group. Concerning diseases, there was a significant difference in distribution between the two groups: acute leukaemia and Hodgkin's disease were more frequent in the IV group whereas chronic myelogenous leukaemia was more frequent in the oral group. However, an impact of disease on PK is very unlikely since there was no significant difference (one-way ANOVA, data not shown) within a group (IV or oral) when comparing Bu pharmacokinetics (clearance or AUC) between the various subgroups of diseases. As a result, data from all diseases within a population group were pooled for further statistical analyses.

Comparison of oral (1 mg/kg) and IV (0.8 mg/kg) Bu AUCs

Direct AUC comparison

First, plasma Bu AUCs following dose 9 were compared between patients who received a fixed dose of oral ($n=71$, 1 mg/kg) and IV Bu ($n=120$, 0.8 mg/kg).

Wider variability with very high AUC values was observed in the oral group as compared with the IV group of patients (Fig. 1). The statistical analysis showed that mean plasma exposure with oral Bu was indeed slightly higher than that with IV. The difference was statistically significant. Nevertheless the bioequivalence test demonstrated that the confidence interval of the AUC ratio was within the acceptance interval for bioequivalence (Table 2).

However, this direct comparison was inappropriate since the body weight index used to calculate the amount of Bu to be administered to patients was different between the two groups. As shown in Fig. 2, the distributions of body weight index (ABW, AIBW or IBW) used for dosing were very different between oral and IV datasets. Dosing for most of the IV Bu patients (68%) was based on IBW, whereas dosing for oral patients was mostly based on either ABW or AIBW.

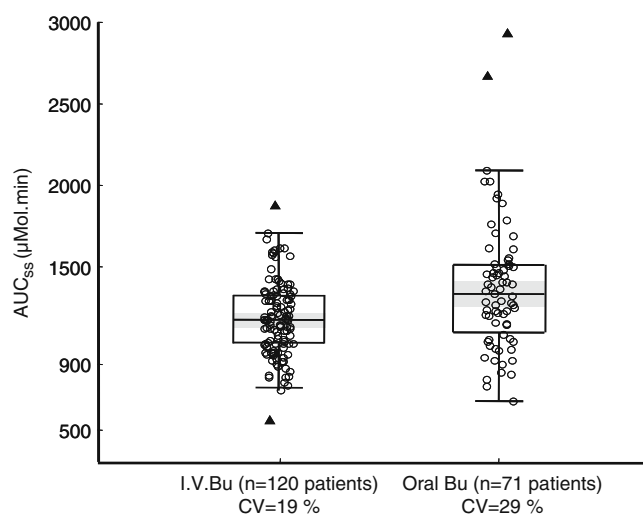


Fig. 1 Box plot of oral AUCs (fixed-dose patients) versus IV AUCs (0–6 h)

Statistical comparisons performed by chi-squared tests confirmed significant differences between IV and oral datasets for each dosing-weight category. Therefore, and even if the same dose level was considered between two datasets, the fact that different body mass indexes were used resulted in different drug amounts administered to patients, which, as a consequence, resulted in different AUCs.

The influence of dosing calculation on the plasma exposure to the drug has already been demonstrated in previous studies on oral [10] and IV Bu [5].

Consequently, datasets were normalised in order to be further compared on the basis of similar dosing weight-index distributions (i.e. AUC normalisation using a similar Bu dose calculation algorithm for oral and IV groups).

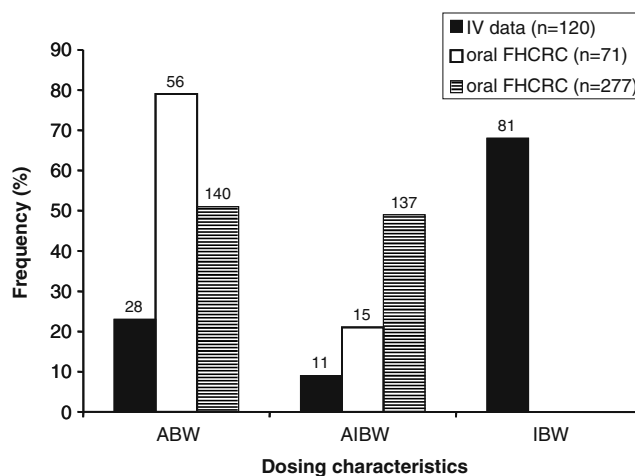


Fig. 2 Comparison of the frequency distribution of body weight indexes used for dosing between the oral and IV datasets

Busulfan exposure assessment based on similar dosing protocols

In order to eliminate the possible confounding influence of the dosing weight-index distributions, IV and oral Bu AUCs were normalised using the same dose calculation method. Since a frequently used and recommended [5, 26] dosing protocol is ABW for patients of normal weight (BMI < 27 kg/m²) and AIBW for obese patients (BMI ≥ 27 kg/m²), all AUC values were normalised according to this dosing protocol.

When based on the same dosing algorithm, a statistically significant difference was no longer observed between oral and IV plasma exposures. Bioequivalence tests showed that the ratio of mean exposure between oral and IV routes was close to unity and that its confidence interval was within the required acceptance range (0.80–1.25) (Table 2 and Fig. 3).

Table 2 Statistical results of steady-state AUC (AUC_{ss}) (0–6 h) comparison following the ninth dose between oral (fixed-dose) and IV patients

	Different dosing-weight protocols		Normalised dosing-weight protocols	
	Oral Bu 1mg/kg	IV Bu 0.8mg/kg	Oral Bu 1mg/kg	IV Bu 0.8mg/kg
Number of patients	71	120	71	120
AUC _{ss} (µM·min)				
Mean ± SD	1,373 ± 400	1,183 ± 228	1,296 ± 362	1,257 ± 242
CV (%)	29	19	28	19
Min	676	556	548	603
Max	2,928	1,874	2,489	2,179
T-Test	P < 0.05		P = 0.72 (NS)	
Ratio of ln(AUC) IV-to-oral	0.878		0.988	
Bioequivalence CI _{90%}	0.830–0.930		0.933–1.05	

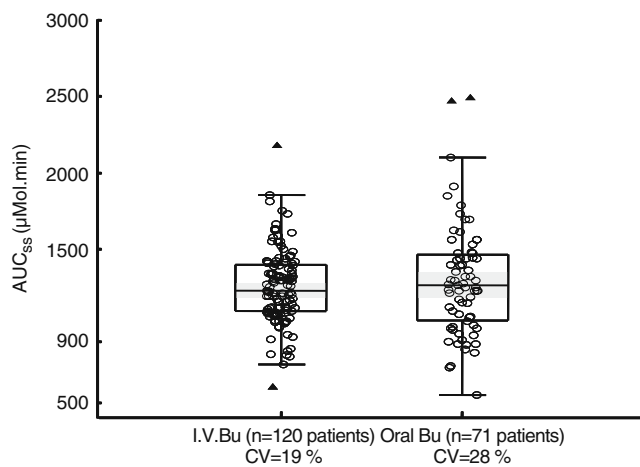


Fig. 3 Box plots of IV and oral Bu AUCs (0–6 h) based on the same dosing algorithm (ABW for BMI < 27 kg/m² and AIBW for BMI ≥ 27 kg/m²)

When using any other dosing protocol (i.e. all oral and IV patients dosed according to ABW, IBW or AIBW), AUC equivalence between 1 mg/kg oral and 0.80 mg/kg IV was also achieved (data not shown).

Estimation of oral Bu absolute bioavailability

Oral and IV comparison was completed on drug clearance values, and average absolute bioavailability was calculated as the ratio of IV to oral clearances.

The analysis on the complete oral dataset ($n=277$ patients) demonstrated that the bioavailability of oral Bu was close to 0.80, resulting in similar blood exposure between 0.8 mg/kg IV and 1 mg/kg oral (Table 3).

Since a statistical difference was previously observed between oral and IV AUCs in the 71 patients from fixed-dose protocols, a similar bioavailability assessment from

clearance analysis was also carried out on this reduced group (Table 3).

Both analyses led to similar results. IV clearance and oral apparent clearance were significantly different. This difference is the consequence of the lower bioavailability of oral versus IV Bu. The clearance ratio indicated an average bioavailability of 0.79 ($n=71$ fixed-dose patients) and 0.83 (all patients), which demonstrated that dose levels equal to 0.8 mg/kg IV and 1 mg/kg oral must be administered to produce similar Bu plasma exposures.

Discussion

Evaluating the absolute bioavailability of an oral drug a few decades after its marketing and following the development of an IV form, is a very uncommon situation. Bioavailability studies are generally conducted early on healthy volunteers whenever possible, based on a randomised cross-over design and evaluated at steady state. For the high-dose Bu conditioning regimen, the situation is complex as the usual schedule consists of 16 doses administered every 6 h over only 4 days. To avoid any chronopharmacokinetic influence [27,28], PK assessments are generally conducted in the morning of days 1, 2, 3 and 4, i.e. at doses 1, 5, 9 and 13. Even if the short half-life of Bu should enable the achievement of steady state at dose 5, it is mostly controlled at dose 9. The first study published by Hassan et al. [3] compared two single Bu administrations: IV at day 1 and oral at day 2. A second study published by Schuler et al. [29] replaced oral dose 2 with an IV dose in the usual 16-dose oral regimen. They compared the PKs at doses 2 and 6, with both at 0.5 mg/kg instead of 1 mg/kg. A third study published by Andersson et al. [20], and concerning the current IV form, substituted the first

Table 3 Average absolute bioavailability assessment for all oral patients calculated based on steady state AUC values from the ninth dose

	All patients		Dose-fixed patients	
	Oral Cl/F (ml.min ⁻¹ .kg ⁻¹)	IV Cl _{total} (ml.min ⁻¹ .kg ⁻¹)	Oral Cl/F (ml.min ⁻¹ .kg ⁻¹)	IV Cl _{total} (ml.min ⁻¹ .kg ⁻¹)
Number of patients	277	120	71	120
Mean ± SD	2.90 ± 0.67	2.41 ± 0.55	3.09 ± 0.86	2.41 ± 0.55
CV (%)	23	23	28	23
Min	1.31	1.42	1.31	1.42
Max	6.07	4.43	6.07	4.43
<i>t</i> -Test	$P < 0.001$		$P < 0.001$	
Bioavailability (F)				
Mean	0.833		0.793	
CI _{95%}	0.794–0.874		0.737–0.852	

dose of the usual 16-dose oral regimen with an IV dose and compared the PK at doses 1 and 5.

These three studies illustrate the difficulty of achieving the three goals of the optimum study design: steady-state, cross-over, randomised. The situation is even more complex when considering the large intra-individual variability of oral Bu PK, in addition to the inter-patient variability. In these three studies, the bioavailability was highly variable, ranging from 11 to 100% (Table 4), and the reliability was limited due to the small sizes of the patient cohorts.

The current retrospective study did not fully meet the criteria of the optimal study design either but may be considered a good compromise. The parallel group design enabled the use of data at steady state for both oral and IV Bu, and the large number of patients from different clinical studies are representative of different clinical uses of Bu.

The development of IV Bu was focused on finding the exact dose that would achieve plasma exposure similar to that of the oral Bu regimen. Through the strong PK/PD established relationship, this finding will enable IV Bu treatment to benefit from the large base of clinical knowledge obtained from oral Bu treatment. In a first study [20], the IV dose was determined in a limited number of patients. The benefit of the current study is to confirm this major issue on large cohorts.

Two different approaches were used in the current study. The first approach relied on a direct AUC comparison using bioequivalence statistics, and the second approach was based on the estimation of oral bioavailability as the ratio of the oral and IV clearances.

Because AUC is dependent on the total administered dose, the comparison between oral and IV forms had to be restricted to patients included in fixed-dosing protocols. Therefore, only oral patients who did not participate in targeting protocols were included in the dose-equivalence evaluation. The direct comparison of AUCs from non-targeted patients demonstrated equivalent Bu AUCs (see Table 1) between 1 mg/kg oral ($n=71$ patients) and 0.8 mg/kg IV Bu ($n=120$ patients).

The oral bioavailability estimated on clearance from the same series of non-targeted patients (71 oral vs. 120 IV) was 0.79 ($CI_{95\%}=0.74-0.85$), demonstrating that the IV dose of Bu should be 80% of the oral dose in order to achieve the same AUC. Since clearance does not depend on the total administered dose, the calculation of bioavailability was extended to the full oral dataset of 277 patients. The value of oral bioavailability was confirmed to be close to 0.80 (mean=0.83, $CI_{95\%}=0.79-0.87$).

Busulfan dosing is generally based on the patient's body weight. In addition to the ABW, several weight indicators are also used for dosing including IBW and AIBW, which are commonly used to avoid over-dosing in obese patients [10]. This reflects clinical practice and has no consequence when comparing large datasets based on similar distributions of dosing-weight indexes. But if the distributions of weight indicators used for dosing are clearly different between two datasets, this may induce a significant difference on dose level comparisons. When different weight-dosing algorithms are used for IV and oral Bu, it may result in a significant difference in Bu AUCs independent of the nominal dose level (0.8 or 1 mg/kg) used for IV and oral administrations. This occurred in the initial direct AUC comparison between IV patients (dosing mainly based on IBW) and fixed-dose oral patients (dosing mainly based on ABW), which resulted in a significant statistical difference between oral and IV AUCs ($P<0.05$ with t -test procedure). The AUC ratio of 0.88 between oral and IV (see Table 2) suggested an oral bioavailability of 0.91, which contrasted with the bioavailability values obtained from clearance ratios (close to 0.80). The latter method illustrated the non-comparable distribution of Bu dosing algorithms used to generate oral and IV datasets. The induced bias produced a statistically significant difference in direct AUC comparisons, although the $CI_{90\%}$ were in the acceptance range. There was no longer a significant difference when the comparison was performed using the same dosing-weight algorithm for all patients (i.e. the frequently used protocol: ABW for patients of normal

Table 4 Literature studies of oral Bu bioavailability in adult patients

Reference	IV Bu formulation	IV Bu dose	Oral Bu dose	Number of patients	Methodology	Bioavailability % (range)
Hassan et al. 1994 [3]	PG/ethanol/DMSO	2 mg total	2 mg total	7	I.V Bu at day 1 and oral Bu at day 2	80 (47–103)
Schuler et al. 1998 [29]	DMSO	0.5–0.6 mg/kg	0.5–0.6 mg/kg	11	Dose 2 (IV) versus dose 6 (oral)	69 (42–94)
Andersson et al. 2000 [20]	DMA / PEG400	0.08–0.8 mg/kg	1 mg/kg	10	Dose 1 (IV) versus dose 5 (oral)	69 (<11–100)

PG Propylene glycol, DMSO dimethylsulfoxide, DMA *N,N*-dimethylacetamide, PEG400 polyethyleneglycol 400

weight and AIBW for obese patients). In that case, AUC ratio was close to unity (from 0.98 to 1.01) and the confidence interval was within the required acceptance interval (0.80–1.25), whatever the dosing protocol or the type of weight index used for dosing normalisation.

Conclusion

The ideal design for bioequivalence and bioavailability assessments of Bu would be a randomised cross-over study at steady-state conditions. In our study, the decreased power induced by the parallel-group design was balanced by the larger number of patients included in the analysis. Both study arms (oral and IV) were comparable in terms of patient demography, treatment characteristics, assay conditions and PK assessment method. AUCs were compared (at steady state) in both groups, excluding any bias that might have existed when using non-steady-state conditions for one arm (i.e. dose 1 versus dose 5 or 9). This favourable situation allowed a powerful statistical comparison and a high probability (calculated power of the test > 99%) to conclude that the 0.8 mg/kg IV Bu and 1 mg/kg oral Bu generate similar exposures.

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