Influence on Busilvex® Pharmacokinetics of Clonazepam Compared to Previous Phenytoin Historical Data

E. CARRERAS[1], J.Y. CAHN[2], C. PUOZZO[3], N. KRÖGER[4], G. SANZ[5], A. BUZYN[6], A. BACIGALUPO[7] and J.P. VERNANT[8]

[1]*Institute of Hematology Oncology, 08036 Bacelona, Spain;*
[2]*Centre Hospitalo-Universitaire, Université Joseph Fourier, 38043 Grenoble, France;*
[3]*Institut de Recherche Pierre Fabre, 31025 Toulouse, France;*
[4]*University Hospital Eppendorf, 20251 Hamburg, Germany;*
[5]*Hospital Universitario La Fe, 46009 Valencia, Spain;*
[6]*CHU Necker, 75743 Paris Cedex 15, France;*
[7]*Ospedale San Martino, 16132 Genova, Italy;*
[8]*CHU Pitié Salpêtrière, 75651 Paris Cedex 13, France*

Abstract. *This study investigated the effect of seizure prophylaxis on busulfan (Bu) plasma exposure. Twenty-four adult patients received an intravenous Bu-cyclophoshamide conditioning regimen prior to bone marrow transplantation. Busilvex® (0.8 mg/kg) was administered every six hours during four consecutive days. Clonazepam (0.025 to 0.03 mg/kg/day as a continuous 12-h i.v. infusion) was administered at least 12 hours prior to i.v. Bu dosing and continued until 24 hours after the last dose. Pharmacokinetic (PK) data were compared with those previously collected in patients (n=127) treated with phenytoin for seizure prophylaxis. Through population PK analysis, a 10% average increase (coeffiecient of variation, RSE=5.35%) in total clearance of Bu was quantified when Bu was associated with clonazepam as compared to phenytoin, which was considered as not being clinically relevant. The suspected induction on Bu metabolism by phenytoin should have resulted in the opposite effect. The patient efficacy and safety profiles were comparable between the two cohorts.*

Alkylating agents are the major class of drugs used in high-dose regimens with bone marrow (BM) or peripheral blood progenitor cells transplantation support (1-7) busulfan (Bu) is a bifunctional alkylating agent that interferes with DNA replication and transcription of RNA and ultimately results in the disruption of nucleic acid (8-11). Due to its poor solubility, Bu was initially developed as an oral form. In adult patients, the combination of high-dose Bu (HDBu), usually 16 oral doses of 1 mg/kg over 4 days and cyclophosphamide (CY) 200 mg/kg or 120 mg/kg (BuCy2) as conditioning regimen, resulted in effective eradication of the host's BM and suppression of the immune response, thereby allowing engraftment by allogeneic hematopoietic stem cell transplantation (HSCT) (3, 12-15). An intravenous form of busulfan has been developed and marketed more recently (Busilvex®) which reduces the inter- and intra-patient exposure variability and its full bioavailability enables estimation of its true pharmacokinetic (PK) parameters (16-18). Since the early use of oral Bu in HSCT, much work has been published on the Bu pharmacokinetic–pharmacodynamic relationship (PK/PD) and an optimal range of Bu plasmatic exposures (AUC), termed therapeutic window, has been established (19, 20) In patients receiving BuCy2 as conditioning regimen, Slattery *et al*. (21) reported the absence of graft rejection with Bu steady-state concentration (Css Bu) >600 ng/ml (*i.e*. AUC>900 μmol. min), whereas Css Bu less than 900 ng/ml was associated with an increased risk of relapse in allo-transplanted CML (chronic myeloid leukemia) patients. Conversely, a high systemic Bu exposure (AUC>1500 mmol.min) increased the occurrence of veno-occlusive disease (VOD), as well as other early serious complications (19-25). Consequently, the optimal therapeutic window has been defined for AUC ranging from 900 to 1500 μmol. min enabling optimal efficacy while minimising post-transplant morbidity and mortality.

Neurological disturbances such as seizures have been reported with the use of HDBu prior to HSCT (20, 26, 27). Busulfan crosses the blood–brain barrier (BBB) readily and

Correspondence to: Christian Puozzo, IRPF, 2 rue Viguerie, BP 3071-31025 Toulouse Cedex 03, France. Tel: +33 562877761, Fax: +33 562877766, e-mail: laurent.nguyen@pierre-fabre.com

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is distributed into the central nervous system (CNS) (28-30). The ratio between cerebrospinal fluid and plasma Bu concentrations is close or higher than 1, and it is presumed that seizures occur as a direct neurotoxic effect (16). As a consequence, prophylactic anticonvulsant therapy is used routinely with Bu-based conditioning regimens (27, 31). Two major chemical categories of drugs are used routinely: phenytoin (PHE) and benzodiazepines (BZDs) (32, 33). They are administered prior to the first dose of Bu until one day after the administration of the last Bu dose. PHE is commonly used in the USA, whereas BZDs are the most common seizure prophylaxis in Europe. PHE is known to be a strong inducer of liver metabolism and is suspected to impact on Bu PK by enhancing its hepatic clearance (34, 35).

During initial Busilvex® drug development, all clinical trials were performed in the USA. The recommended dose and the safety profile were thus determined in patients who all received PHE as seizure prophylaxis. If PHE co-treatment had induced Bu metabolism enzymes and thus had accelerated Bu elimination, an overexposure to busulfan would have been observed in Europe where BZDs are extensively used instead of PHE.

The purpose of the present study was to evaluate Bu exposure when combining clonazepam seizure prophylaxis with Busilvex® conditioning regimen and to compare these PK data with historical results obtained when PHE was co-administered with Busilvex®.

Patients and Methods

Study design. This study was a prospective multicentre, open-label, uncontrolled study with a standard *i.v.* BuCy2 regimen for HSCT in adult patients with haematological malignancies. Nine clinical centres in four European countries were involved. The study was authorized by the National Health authorities of each country according to legal requirements, and the protocol was approved by the local Ethics Committees before the inclusion of patients. The study was performed in accordance with the principles stated in the Declaration of Helsinki and in accordance with the European Good Clinical Practice guidelines. Patients received Busilvex® (*i.v.* Bu, 0.8 mg/kg) every six hours during four consecutive days, one day of rest and then Cy (60 mg/kg/day) during two consecutive days, followed by one day of rest, and stem cell infusion (HSCT day 0). *i.v.* Bu was administered at a fixed dose without therapeutic drug monitoring and dose adjustment, according to what is recommended in the SmPC (36). For seizure prophylaxis, clonazepam (Rivotril®, 0.025 to 0.03 mg/kg/day as a continuous 12-h *i.v.* infusion) was administered at least 12 hours prior to *i.v.* Bu dosing and until 24 hours after the last dose.

The population sample size (20 evaluable patients) was determined using the one group *t*-test to compare a mean theoretical value to an observed value. Assuming that value to be clinically significant, the expected difference between the reference mean total clearance Cl_{tot} value (PHE treatment group) and the observed mean Cl_{tot} value (BZD treatment group) must be at least 10-15%. The expected inter-individual variability previously determined by population approach, and associated with the reference mean Cl_{tot} value, ranged from 15% to 20%. First type (alpha risk) and second type (beta risk) errors were set at 5% and 20% (power=80%), respectively.

Patient selection. Adult patients were eligible to enter in the study if they had established haematological malignancies planned to receive the BuCy2 conditioning regimen before HSCT from HLA-matched (10/10) or mismatched (9/10) related or unrelated donors. The main inclusion criteria were: age (18-55 years), Karnofsky's performance status (≥70%), adequate organ system function (cardiac, pulmonary, hepatic and renal) and signed informed consent after full explanation of the study. A patient was considered evaluable for PK analysis if complete blood sampling was achieved at least at dose number 9.

Pharmacokinetics. PK analyses were performed in all patients at doses 1, 5, 9 and 13 in order to estimate Bu PK parameters using a limited sampling strategy (LSS) and a population PK model (18). Plasma samples were obtained from a peripheral vein that was not used for Bu administration and collected at pre-dose, 2.5 and 6 h post Bu dosing. Samples were centrifuged and stored at –20˚C until bioanalysis. Bu plasma concentrations were determined using a gas chromatography-mass spectrometry (GC-MS) method with a limit of quantification set at 62.5 ng/ml (37). The within- and between-run coefficients of variation were below 10%. Development of the population PK model for the reference group (PHE group) was based on PK data from 127 autologous and allogeneic transplant adult patients with samples drawn at Bu dose numbers 1, 9 and 13 (18). In all these studies, the preparative regimen for engraftment was similar. All patients received a standard 16-dose regimen of *i.v.* Bu (0.8 mg/kg) administered every 6 hours over 4 days. This treatment was followed by a standard Cy regimen. Phenytoin was used for anti-seizure prophylaxis. The pharmacokinetic data of the present study (BZD group) were merged with the historical pharmacokinetic data (*i.e.* PHE group) and the whole data set was reprocessed using the NONMEM® program (Version 6.0) (38). Individual *i.v.* Bu plasma exposures (AUC_{inf}) were calculated at dose numbers 1, 5, 9 and 13 through Bayesian methodology (POSTHOC option in NONMEM® program). The two covariates included in the reference model were BSA (body surface area) and ABW (actual body-weight), and the anticonvulsant prophylaxis (BZD or PHE) was tested as an additional covariate. Evidence of a statistical difference was determined by a decrease in the objective function value (OFV) from the reference model. The magnitude of the difference between the two groups was considered as clinically relevant if it was higher than or equal to 20%. For the precision of the computed magnitude, a coefficient of variation (RSE) lower than 30% was required to reach a firm conclusion. The comparison was also performed using individual empirical Bayesian estimates of Bu Cl_{tot} at dose number 9, and a Wilcoxon's test between the two treatment groups was carried out since Bu Cl_{tot} was not normally distributed.

Pharmacokinetic/pharmacodynamic analysis. Bu exposures were compared between the patients who experienced VOD and patients who did not.

Clinical assessments. Safety and efficacy were assessed throughout the study treatment period defined by the protocol as 37 days from HSCT day –9 through HSCT day +28 and during post-study surveillance (HSCT day +29 through HSCT day +100). Toxicities were graded according to NCI/CTC Version 2.0 scale (39). All

patients having received at least one dose regimen of *i.v.* Bu were included in the clinical assessment analyses including also frequency and severity of regimen related toxicities (RRT) affecting seven organ systems which included heart, bladder, kidney, breast, liver, central nervous system and gastro -intestinal tract.

Results

Twenty-four out of 25 patients with various haematological malignancies planned to receive HSCT were evaluable for PK and safety. One patient was included but not treated due to severe cardiac function abnormalities and this patient moved to an alternative treatment regimen. Two protocol deviations occurred and were due to one obese patient who was dosed with Bu according to ABW instead of Adjusted ideal body weight (AIBW) and one normal-weight patient with the opposite situation. Nevertheless, both patients were considered evaluable.

Patient characteristics. The median age of the 24 patients, 15 males and 9 females, was 45.6 years (range: 18.1-56.5 years). The baseline and disease characteristics, as well as the status at transplantation are outlined in Table I. Twenty-one patients presented myeloid malignancies and three presented lymphoid malignancies. They constituted a heavily pre-treated group with 46% of the patients in CR1, and 50% having active disease at HSCT. A comparison of the baseline characteristics between the current study patients and the reference patients (PHE group) is presented in Table II. Demographic characteristics between the two groups were comparable, except for obese patients that were slightly more frequent in the PHE group.

Pharmacokinetics. Individual plasma Bu concentrations (n=133) *versus* time of the current study (BZD group) were consistent with those collected in the PHE group (Figure 1). The parameters of the *i.v.* Bu PK model previously developed in adult patients (18) were re-estimated on the full dataset by merging together the current data (n=24 patients) with the reference data (n=127 patients). Parameters were estimated with a good precision (low relative standard errors) and were consistent with those already obtained with the reference dataset, indicating that the model was stable. Individual PK parameters (empirical Bayesian estimates) were calculated from the refined model (n=151 patients) for each Bu dose explored. Plasma Bu exposures were very similar between the two groups at dose 1, but were higher in the PHE group than in the BZD group at doses numbers 9 and 13 (Table III). Thus in order to assess the degree of significance of this apparent difference, the type of seizure prophylaxis was introduced as a covariate in NONMEM ('BZD covariate'). However when including this BZD covariate, the model parameters were not modified and the objective function was only reduced by less than 3.84.

Table I. *Demographics and baseline characteristics*.

Patient characteristic (n=24)	Value
Age (years)	
Median	45.6
Range	18.1-56.5
Gender	
Male	15
Female	9
Acute myeloid leukaemia (AML)	12 (50%)
CR1	10
CR2*	1
Untreated relapse	1
Myelodysplastic syndrome	4 (17%)
RAEB	2
RAEB/CR1	1
CMML	1
Chronic myelogenous leukaemia	5 (21%)
CP	4
AP	1
Lymphoma	3 (12%)
NHL	2
Progressive	1
Refractory	1
HD	1
PR2	1

*Patient with AML3, AP: Accelerated phase, CMML: chronic myelomonocytic leukaemia, CP: chronic phase, HD: Hodgkin's disease, NHL: non-Hodgkin's lymphoma; RAEB: refractory anaemia with excess of blasts.

Consequently, the contribution of this covariate was not statistically significant. Moreover, the BZD effect on Bu clearance was estimated with a high precision (standard error close to 5%) from the model and indicated that the difference between PHE and BZD groups was about 10%. This difference was not considered clinically relevant as defined by the protocol criteria (<20%). After the 9th administration, a steady state was assumed to be achieved and individual empirical Bayesian estimates of Bu Cl_{tot} were further compared between PHE and BZD groups using Wilcoxon's test. The mean (±sd, standard deviation) Bu Cl_{tot} values were 14.1± 3.1 l/h and 11.2±2.7 l/h in the BZD and PHE groups, respectively, and were significantly different ($p<0.0001$) (Table III). The inter-patient variability in Cl_{tot} estimated from the refined population PK model (151 patients) was 18.2% (RSE=17.0%), while the inter-occasion variability (inter-administrations) was 11.5% (RSE=16.8%). The inter-patient variability calculated from the current study data (BZD group) was very similar, with coefficient of variation (CVs) for Bu Cl_{tot} ranging from 18% at dose number 1 to 22% at dose number 9 (Table III). The intra-patient variability calculated from the Bu Cl_{tot} variance analysis (two-way, patient and dose) was about 6%.

Table II. *Comparison of baseline characteristics between study patients (BZD group) and reference patients (PHE group).*

Group	n	Parameter	Mean	Median	sd	Minimum	Maximum
		Age (years)	38.8	38.0	11.6	14.3	64.0
		Height (cm)	171	170	9.6	148	198
		BSA (m^2)	1.9	1.9	0.3	1.4	2.5
		Actual body weight (kg)	77.4	76.0	18.7	41.0	125
PHE	127	Ideal body weight (kg)	65.3	64.6	10.7	41.4	91.9
		Adjusted ideal body weight (kg)	68.3	38.2	11.1	47.2	95.1
		Body mass index (kg/m^2)	26.3	25.2	5.7	15.3	46.9
		Gender (male /female)			72 (57%)/55 (43%)		
BZD	24	Age (years)	43.4	45.6	11.7	18.1	56.5
		Height (cm)	173	172	9.1	160	194
		BSA (m^2)	1.8	1.9	0.2	1.5	2.2
		Actual body weight (kg)	72.4	72.0	12.6	52.0	96.0
		Ideal body weight (kg)	67.4	68.2	10.0	82.3	88.2
		Adjusted ideal body weight (kg)	68.6	70.3	9.8	52.9	87.3
		Body mass index (kg/m^2)	24.1	23.4	3.4	19.5	32.3
		Gender (male /female)			15 (63%)/9 (37%)		

sd: Standard deviation.

Table III. *Descriptive statistics of i.v. Bu pharmacokinetic parameters.*

Group	Bu dose no.	n	Variable	Mean±sd	Median (range)	CV%
Reference	1	114	AUC (μmol min)	1060±208	1062 (530-1632)	20
			Cl_{tot} (l/h)	12.5±3.0	12.3 (5.4-23.5)	24
	9	121	AUC (μmol min)	1177±227	1170 (540-1858)	19
			Cl_{tot} (l/h)	11.2±2.7	10.6 (6.1-23.0)	24
	13	109	AUC (μmol min)	1207±250	1184 (718-1910)	21
			Cl_{tot} (l/h)	11.1±2.7	11.0 (5.2-18.0)	24
Study	1	23	AUC (μmol min)	1003±178	967 (739-1458)	18
			Cl_{tot} (l/h)	14.0±2.5	13.1 (8.8-18.6)	18
	5	24	AUC (μmol min)	965±190	928 (680-1394)	20
			Cl_{tot} (l/h)	14.8±3.2	14.6 (8.4-23.3)	22
	9	24	AUC (μmol min)	1023±237	989 (756-1731)	23
			Cl_{tot} (l/h)	14.1±3.1	13.9 (7.1-20.7)	22
	13	23	AUC (μmol min)	1084±246	1026 (817-1830)	23
			Cl_{tot} (l/h)	13.1±2.7	12.5 (8.4-18.4)	21

sd: Standard deviation, CV: coefficient of variation, AUC: area under the curve, Cl_{tot}: total clearance.

Safety. i.v. BuCy2 conditioning regimen concomitantly administered with BZD as seizure prophylaxis was well tolerated. No adverse events (AEs) were observed during *i.v.* Bu administration and no patient developed seizure while receiving *i.v.* Bu. Neither new nor unexpected toxicities were reported and neither severe nor mild/moderate RRT were fatal or necessitated to discontinue the study treatment. Two allogeneic recipients had reversible VOD (*i.e.* 8% incidence). Twenty-one serious adverse events (SAEs) were reported in all patients treated. Nine and twelve SAEs occurred during the study period and during the short-term post-study surveillance, respectively. The SAEs were those usually observed and expected with the BuCy2 conditioning regimen (16, 36, 40-42). One SAE was fatal in the context of severe uncontrolled acute GVHD (24, 43-45). Early transplant-related mortality at HSCT day 100 was 4% (95% CI: 0.1-21). This was a low rate in this high-risk population. In the current study, all patients engrafted (100%) with sustained engraftment in all but one as documented by chimera data. Furthermore, neither early nor late graft rejections were reported. For one patient, the reversion of chimerism preceded recurrence of the underlying disease (data not shown).

Discussion

The use of HDBu-based conditioning regimen and the capacity of Bu to cross the BBB necessitate a prophylactic treatment to avoid the occurrence of seizures. Two major classes of drugs are generally used: PHE and BZD (32, 33). Concerning the first class, PHE is metabolised through the cytochrome P450 system and is known to be a strong inducer of liver metabolism (35). Therefore PHE is suspected to impact on Bu pharmacokinetics by enhancing Bu hepatic clearance, which should affect Bu exposure and, as a consequence, modify its clinical activity and toxicity. Nevertheless, the mechanism of PHE impact on Bu metabolism appears questionable since PHE is not clearly described as an inducer of glutathione-*S*-transferase (GST) enzymes, the major component involved in Bu metabolism (11, 28, 29, 46, 47). More specifically, PHE increases the metabolism of drugs which involve CYP2C, CYP3A and uridine diphosphoglucuronyl transferase (UDPGT) (35, 48, 49). The second class, BZDs, include drugs such as clonazepam, diazepam, clobazam and lorazepam, and have been successfully used as prophylactic anticonvulsant therapy (4, 27, 50, 51). The initial development of Busilvex® (*i.v.* Bu), which included the evaluation of the maximal tolerated dose during phase I trial, was conducted in the USA and PHE was used as seizure prophylaxis (40, 41). Consequently, the further use of BZDs as seizure prophylaxis may have resulted in a Bu overexposure which may have induced severe clinical consequences (19, 20, 23-25). The current study was designed to assess the impact of moving from PHE to BZDs seizure prophylaxis. Clonazepam was chosen in the present study because it is one of the most commonly used BZD in Europe. The Bu conditioning regimen administered over 4 days with a seizure prophylactic treatment generally starting one day before precluded a powerful cross-over design to compare PHE and BZD effects on the same patient. The duration of PHE administration was too short to achieve a real induction. As a result, the influence of seizure prophylaxis had to be evaluated on a parallel group design. Retrospective data and the same bioanalytical technique were used in the current study and in the previous historical data.

Several investigators described the potential effects of PHE on the metabolism of Bu (30, 52, 53) but literature data from oral Bu PK are controversial and fail to provide consistent and conclusive results on the potential effect of PHE or BZD (54). This could be partly due to the limited number of patients enrolled in the studies, but is more likely the consequence of the high variability of oral Bu PK hampering a clear-cut conclusion. Hassan *et al*. (30). compared Bu metabolism in two groups of patients treated with either PHE (n=9) or diazepam (n=8) as anticonvulsant prophylaxis. Patients who received PHE demonstrated a significantly higher clearance, a lower AUC at dose 1 *versus* that at dose 16 (1577±543 μmol min *versus* 1318±374 μmol min, $p<0.05$) and a shorter elimination half-life following the last Bu dose, while no modification was observed in the diazepam group. Embree *et al*. (55). evaluated whether PHE (n=6) or diazepam (n=6) can interfere with either the initial or the steady-state PK of oral Bu. The authors did not detect any statistical difference between the two treatment groups and concluded that the PHE induction of P450 enzymes does not alter the PK of Bu-based conditioning regimens. Conversely, the situation with BZD is clearer. No interactions with Bu metabolism were demonstrated in several clinical studies with oral Bu-based conditioning regimens prior to HSCT when BZDs such as diazepam, clobazam, clonazepam and lorazepam were used (4, 30, 50).

In the current study, it was hypothesised that the administration of clonazepam (Rivotril®) would not alter Bu PK and its pharmacodynamics. The current data demonstrated that *i.v.* Bu PK was comparable in patients who received either BZD or PHE. The effect tested on Bu Cl_{tot} in NONMEM was not statistically significant (10% mean difference between PHE and BZD groups) and not clinically relevant as defined by the protocol (<20%). Further comparison of the clearance values between BZD and PHE groups at dose number 9 showed slightly higher values in the BZD group (Wilcoxon's test, $p<0.01$), even though the range of values between the two groups overlapped. However, such difference would indicate the opposite effect to that which was expected. An induction of Bu elimination by PHE prophylaxis would have led to higher Bu Cl_{tot} in the PHE group whereas only slightly lower values were observed in this group in the current study. These findings indicate that PHE does not induce Bu metabolism. Therefore, when BZD are used instead of PHE for seizure prophylaxis, the dose correspondence between oral and *i.v.* Bu dosing must be the same, *i.e.* 1.0 mg/kg *versus* 0.8 mg/kg, respectively. Concerning the *i.v.* Bu PK, the current study confirmed the low inter-patient and intra-patient variabilities. The inter-patient variability on Bu Cl_{tot} ranged from 18% at dose number 1 to 22% at dose number 9. The average intra-patient variability was about 6%. These values are similar to those previously collected in patients with PHE prophylaxis in the US trials (17, 18, 40, 41).

Concerning the safety, *i.v.* BuCy2 combined with BZD as seizure prophylaxis was well tolerated. No seizure was observed during Bu treatment. As expected, the *i.v.* BuCy2 regimen was frequently associated with elevated liver enzymes, but VOD was infrequent (n=2), with mild/moderate severity, and resolved without sequelae in both patients. VOD was not related to high Bu AUCs, confirming that this toxicity is multifactorial (56-59). Nevertheless and even though many risk factors are involved in the development of VOD as described in the literature (60-64), the control of a major factor (namely Bu overexposure) should reduce the overall risk and assure good efficacy, as reported in the current study.

Conclusion

Busulfan blood exposure was evaluated with concomitant administration of BZD as seizure prophylaxis in adult patients with haematological malignancies receiving *i.v.* BuCy2 regimen, and retrospectively compared with that observed in a reference population using PHE as a prophylactic treatment. The evaluation of BZD co-administration effect on Bu Cl_{tot} was performed using a population PK methodology which enabled a powerful analysis to test the significance of the effect. No relevant difference was detected between the two groups and the inclusion of seizure prophylaxis treatment as a covariate in the model was not statistically significant. Therefore, no Bu overexposure is expected when administering BZDs as seizure prophylaxis instead of PHE and this result was further supported by the clinical data. In conclusion, clonazepam can be safely used as an alternative for adequate anticonvulsant prophylaxis during *i.v.* Bu treatment.

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