

Drug Interactions

In children and adolescents, the pharmacodynamics of high-dose busulfan is dependent on the second alkylating agent used in the combined regimen (melphalan or thiotepa)

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Summary:

A strong relationship has been demonstrated between high systemic exposure to busulfan and the occurrence of hepatic veno-occlusive disease (HVOD) after a busulfan–cyclophosphamide regimen (BU CY). We report a prospective study aimed at exploring the pharmacodynamics of high-dose busulfan combined with either melphalan (BU MEL) or thiotepa (BU TTP) followed by autologous stem cell transplantation in children and adolescents with a malignant solid tumor. Busulfan was given orally at a total dose of 600 mg m⁻². In all, 45 patients with a median age of 6.3 years were included in the study: 25 received BU MEL and 20 received BU TTP. The incidence of HVOD was 44% (CI 95% [23–65%]) in the BU MEL group and 25% (CI 95% [9–49%]) in the BU TTP group. In the BU TTP group, patients who developed HVOD had a significantly higher AUC 0–6 h after the 13th dose (6201 ± 607 h ng ml⁻¹) than those who did not (5024 ± 978 h ng ml⁻¹) (*P* < 0.05). In the BU MEL group, there was no difference in terms of systemic exposure to busulfan between patients who developed HVOD and those who did not. In conclusion, the guidelines established for monitoring BU CY cannot be extrapolated when busulfan is combined with another drug.

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Busulfan (1,4-butanediol dimethanesulfonate) is an alkylating agent with both myeloablative and antitumor properties¹. High-dose busulfan is widely used in combination

chemotherapy regimens followed by allogeneic or autologous hematopoietic stem cell transplantation (HSCT) for the treatment of several diseases, including leukemias, solid tumors and genetic diseases. When combined with cyclophosphamide, busulfan is an efficient myeloablative regimen before allogeneic stem cell transplantation and used in the treatment of several bone marrow (BM) disorders and leukemias.^{2–5} Busulfan-containing regimens followed by autologous HSCT have also been developed for the treatment of malignant solid tumors, especially in children and adolescents. Busulfan–melphalan (BU MEL) is an active treatment for high-risk Ewing's tumors and neuroblastoma, and is currently used as consolidation treatment for minimal residual disease in these poor prognosis diseases.^{6,7} In addition, we have shown that busulfan–thiotepa (BU TTP) is an effective salvage therapy for relapsed medulloblastoma in children.^{8,9}

Hepatic veno-occlusive disease (HVOD)^{10,11} is the most severe and frequent extrahematological toxicity associated with high-dose busulfan in both adults and children.^{12,13} Over the last 15 years, tremendous efforts have been expended to evaluate whether drug monitoring and dose adjustment could reduce the incidence and severity of busulfan-containing high-dose chemotherapy. It is now well established that the spectrum of interpatient and inpatient variability of busulfan disposition is wide, both in adults and in children.¹⁴ Moreover, a significant relationship has been demonstrated between high systemic exposure to busulfan during a busulfan–cyclophosphamide (BU CY) regimen and the occurrence of severe toxicity.^{13,15} Slattery *et al*¹⁶ showed that exposure to busulfan corresponding to a steady-state concentration (*C*_{ss}) of between 600 and 900 ng ml⁻¹ guaranteed both engraftment and a low incidence of HVOD in adult patients undergoing allogeneic HSCT after BU CY. In that study, *C*_{ss} was defined as the area-under-the-curve (AUC_{0–6 h}) at steady state divided by 6 h, that is, the time interval between two busulfan ingestions. When converted into the AUC, this therapeutic window ranges from 3600 to 5400 h ng ml⁻¹. Recently, this therapeutic window was shown to be relevant in children undergoing allogeneic HSCT after BU CY.¹⁷ Pharmacokinetically guided dose adjustment for BU CY is being performed and explored by several teams.^{18–21}

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In contrast, we have previously reported the absence of such a pharmacodynamic relationship when busulfan is combined with other alkylating agents.²² In that previous study, several high-dose busulfan-containing regimens were evaluated and 39 out of 61 patients received three alkylating agents, namely busulfan, cyclophosphamide combined with either melphalan or thiotepa. The number of alkylating agents during busulfan containing high-dose chemotherapy was an independent risk factor for the occurrence of HVOD in children¹² and it may have jeopardized the likelihood of identifying a relationship between high exposure to busulfan and toxicity. To further evaluate the need for busulfan drug monitoring in regimens other than BU CY, we designed a prospective study to explore the pharmacodynamics of high-dose busulfan in pediatric patients receiving either BU MEL or BU TTP high-dose chemotherapy.

Patients and methods

Patients

Plasma busulfan pharmacokinetics were prospectively studied in 45 patients (18 girls and 27 boys) with a median age of 6.3 years (range, 1–20 years) in a single institution. Patients were treated for a malignant solid tumor with either busulfan and melphalan (25 patients) or busulfan and thiotepa (20 patients) followed by autologous hematopoietic stem cell support (Table 1). One patient had a

45% reduction in the glomerular filtration rate, as evidenced by ⁵¹Cr-EDTA clearance. All other patients had normal liver and kidney functions before treatment. The median number of prior conventional chemotherapy lines was two for the BU MEL group and one for the BU TTP group ($P < 0.0001$). BU MEL was administered in treatment of neuroblastoma ($n = 8$), Ewing's tumor ($n = 15$) and peripheral PNET (primitive neuroectodermal tumor, $n = 2$). BU TTP was prescribed for the treatment of central nervous system tumors, that is, medulloblastoma ($n = 13$), ependyoma ($n = 5$) and central PNET ($n = 2$). All tumors were histology proven. Parents and adult patients had given their consent.

Treatment

Busulfan was always the first drug administered. It was given orally every 6 h over 4 consecutive days at a total dose of 600 mg m^{-2} . A patient previously allografted, after total body irradiation and cyclophosphamide for acute lymphoblastic leukemia, received a reduced dose of busulfan (400 mg m^{-2}). Treatment was started at 0012 h for all patients. Patients received nothing *per os* (except water) 2 h before and 30 min after ingestion. There was always a 30 h time interval between the last intake of busulfan and the administration of the second alkylating agent, melphalan or thiotepa. Melphalan was given as a single short 20-min i.v. infusion, at a dose of 140 mg m^{-2} . Thiotepa was given as a 1 h i.v. infusion at a daily dose of 300 mg m^{-2} over 3 consecutive days. All patients received clonazepam in a

Table 1 Patient characteristics

BU MEL group (n = 25)							Bu TTP group (n = 20)						
Patients	Sex	Age (years)	Disease	Prior CT (n)	AUC _{0-6h} (hng ml ⁻¹)	HVOD GRADE	Patients	Sex	Age (years)	Disease	Prior CT (n)	AUC _{0-6h} (hng ml ⁻¹)	HVOD GRADE
1	F	3.8	NB	4	4925	0	26	M	1.8	MB	1	5762	0
2	F	13.1	PNET	2	5679	1	27	F	8	PNET	1	4247	0
3	F	12.1	EWS	3	3313	0	28	F	10.5	MB	2	4723	0
4	M	2.1	NB	3	3906	2	29	M	2.7	MB	1	5568	0
5	F	2.1	NB	3	4359	0	30	M	6.3	MB	1	5966	0
6	M	12.3	EWS	2	7315	0	31	F	9.9	MB	2	6026	1
7	M	4.8	NB	3	4800	0	32	F	3.4	EP	1	6216	0
8	F	13.6	EWS	3	3190	1	33	F	1.1	EP	1	6488	2
9	M	20	EWS	2	3761	2	34	M	5.8	EP	1	7052	0
10	F	2.1	NB	2	6795	1	35	M	15.7	EP	1	3776	0
11	M	14	EWS	3	5754	3	36	F	2	MB	1	4553	0
12	F	17.4	EWS	3	5594	0	37	M	3	MB	1	4812	0
13	M	2.4	EWS	2	7287	0	38	M	9.7	PNET	1	5378	0
14	M	2.7	NB	3	2314	1	39	M	2.9	EP	1	4325	0
15	M	19.8	EWS	2	4510	2	40	M	3.2	MB	2	4516	0
16	M	14.6	EWS	3	3191	2	41	M	4.8	MB	1	6067	3
17	F	19.4	EWS	2		0	42	M	5.3	MB	1	3369	0
18	M	11.8	EWS	2	6129	0	43	F	3.5	MB	1	5095	0
19	M	11.5	EWS	2	4754	0	44	M	13.5	MB	1	5392	3
20	M	14.5	EWS	2	5744	0	45	F	3.9	MB	1	7032	3
21	M	8.2	EWS	1	4597	0							
22	M	1.2	NB	2	4437	2							
23	F	3.2	NB	3	4573	0							
24	M	7.3	Lymph	1	4738	3							
25	F	6.4	EWS	2	5748	0							

NB, neuroblastoma; PNET, primitive neuro-ectodermal tumor; EWS, Ewing's tumor; lymph, lymphoma; MB, medulloblastoma; EP, ependyoma; CT, conventional chemotherapy; HVOD, hepatic veno-occlusive disease; AUC_{0-6h}, area-under-the-curve at steady state after the 13th dose.

continuous i.v. infusion as prophylaxis against busulfan-induced seizures throughout the course of treatment. High-dose chemotherapy was delivered along with hyperhydration (31m^{-2}). Autologous stem cell transplantation was performed 24h after the last dose of melphalan or thiotepa. In all, 32 patients received BM, 10 patients received peripheral blood stem cells (PBSC) and two patients received both BM and PBSC. Patients were in single laminar air-flow rooms.

Liver toxicity grading

The diagnosis of HVOD was based on McDonald's criteria.²³ At least two of the following three abnormalities had to be fulfilled: bilirubin $> 34.2\ \mu\text{mol l}^{-1}$; hepatomegaly or liver pain; weight gain of at least 5% and/or ascites. The severity of HVOD was defined as follows: moderate disease not requiring treatment (grade 1); mild disease with hydic retention requiring treatment (grade 2); severe disease that failed to resolve after 100 days or whose outcome proved fatal (grade 3).

Blood sampling and busulfan assay

Whole-blood samples were drawn through a central venous line. Samples were obtained 0.5, 2 and 6 h after the first dose of busulfan and 6 h after the second, third, fourth, 12th and 13th dose to measure trough plasma levels (Figure 1). Complete pharmacokinetic sampling was obtained after the 13th dose, that is, at 0.5–1–2–3–4 and 6 h after drug ingestion. Samples were immediately centrifuged at 4°C for 10 min at 3000 rpm. The plasma was frozen and stored at -80°C until assay. Plasma busulfan concentrations were determined using a gas chromatography and mass spectrometry assay with a deuterated internal standard busulfan-d₄, as previously reported.²⁴

Pharmacokinetic analysis

The AUC extrapolated to infinity according to the elimination phase ($\text{AUC}_{0-\infty}$) was estimated after the first dose using our previously published limited sampling model²⁵ and according to the following equation: $\text{AUC}_{0-\infty} = 122 + 0.97C_{(0.5\text{h})} + 13.94C_{(6\text{h})}$, where $C_{(0.5\text{h})}$ and $C_{(6\text{h})}$ are the plasma levels measured 0.5 and 6 h after dosing. The plasma level measured 2 h after the first dose, that is, close to the T_{max} , was used as a quality control parameter in order to identify patients who were not in the elimination phase 6 h after drug intake. The steady-state $\text{AUC}_{0-6\text{h}}$ after the 13th dose was calculated using the trapezoidal rule with the PKCalc program²⁶ from time 0 to 6 h after administration. The total clearance at steady state was calculated by dividing the administered dose by the $\text{AUC}_{0-6\text{h}}$.

Statistical analysis

Proportions were compared using the χ^2 test. The Mann–Whitney and Wilcoxon and Friedman nonparametric tests were used to compare values.

Results

Hepatic veno-occlusive disease (HVOD)

Among the 45 children treated with BU MEL or BU TTP, 16 developed HVOD (36%, $\text{CI}_{95\%}$ [22–50%]) according to McDonald's criteria. The incidence was not significantly different between boys (37%) and girls (33%). In the BU MEL group, 11 out of 25 patients (44%, $\text{CI}_{95\%}$ [23–65%]) developed HVOD. Five patients had grade 2 disease and two patients grade 3 disease. One patient died of HVOD. In the BU TTP group, five out of 20 patients (25%, $\text{CI}_{95\%}$ [9–49%]) developed HVOD. One patient had grade 2 disease and three patients grade 3. One patient died of HVOD. The incidence of HVOD was higher in the BU

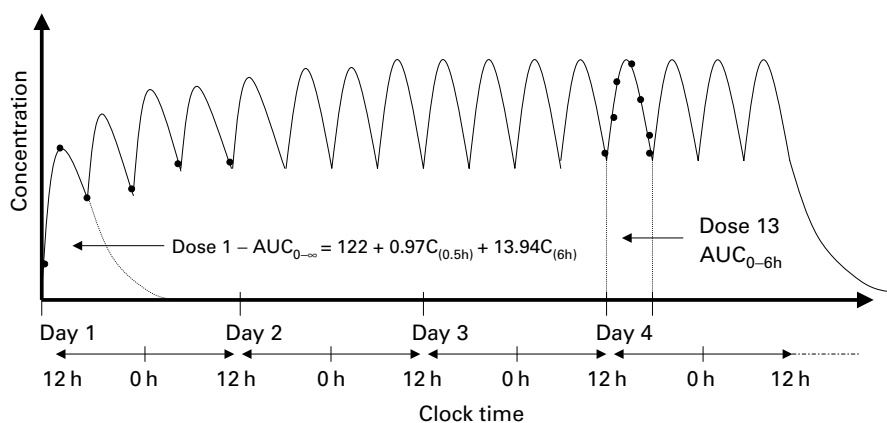


Figure 1 Pharmacokinetic sampling. Busulfan was given orally every 6 h over 4 consecutive days. Treatment was started the first day at 12 h. The circles represent the sampling times during the 4 days of treatment: 30 and 120 min after the first ingestion, and just before the following ingestion at 18, 0, 6 and 12 h. Seven successive measurements were made on the fourth day, from 12 to 18 h. The solid line simulates the assumed evolution of busulfan concentrations over the 4 days of observation. The AUC extrapolated to infinity according to the elimination phase ($\text{AUC}_{0-\infty}$) was estimated after the first dose using our previously published limited sampling model and according to the following equation: $\text{AUC}_{0-\infty} = 122 + 0.97C_{(0.5\text{h})} + 13.94C_{(6\text{h})}$, where $C_{(0.5\text{h})}$ and $C_{(6\text{h})}$ are the plasma levels measured 0.5 and 6 h after dosing. The steady-state $\text{AUC}_{0-6\text{h}}$ after the 13th dose was calculated using the trapezoidal rule.

MEL group (11/25) than in the BU TTP group (5/20), but the difference was not statistically significant ($P=0.19$).

Busulfan disposition

In both regimens, busulfan was the first drug administered in standardized conditions. No difference was observed between the two treatment groups with regard to the AUC_{0-6h} at steady state and the mean trough plasma busulfan levels. All the pharmacokinetic data were therefore analyzed together.

After the first dose of busulfan, six patients had a trough plasma level 6 h after drug intake that was greater than the plasma level at 2 h. Consequently, an $AUC_{0-\infty}$ could be estimated using the equation 1 given above only in 38 out of 45 patients (84%) and it ranged from 1105 to 8084 $h\ ng\ ml^{-1}$.

In keeping with the study design, complete pharmacokinetic sampling was obtained and evaluated after the 13th dose, that is, at steady state, in 44 patients. Plasma samples were not collected for one patient. The mean \pm s.d. total clearance uncorrected for bioavailability (CI/F) was $127 \pm 76\ ml\ min^{-1}\ m^{-2}$ and showed a 6.6-fold interpatient variation ranging from 47 to 313 $ml\ min^{-1}\ m^{-2}$. The AUC_{0-6h} ranged from 2314 to 7315 $h\ ng\ ml^{-1}$ with a mean \pm s.d. of $5086 \pm 1183\ h\ ng\ ml^{-1}$. These values are in good agreement with the pharmacokinetic parameters already reported in children receiving oral busulfan.²⁷⁻²⁹ Moreover, there was a poor correlation between these measured AUC_{0-6h} at steady state and the estimated $AUC_{0-\infty}$ after the first dose ($r^2=0.28$). That is why we decided to continue the pharmacodynamic analysis using only the AUC_{0-6h} measured at steady state (ie after the 13th dose with a full pharmacokinetic sampling).

Trough plasma levels were obtained in 37 patients over 24 h during the first day of treatment, and after doses 12 and 13. The statistical analysis on paired data confirmed the reported within-day variations by showing that trough levels differed significantly over a 24-h period (Friedman's test, $P<0.0001$). The main differences were observed between busulfan trough levels at 1800 h with a mean \pm s.d. of $596 \pm 257\ ng\ ml^{-1}$ and at 0600 h with a mean \pm s.d. of $406 \pm 139\ ng\ ml^{-1}$ (Dunn's multiple comparison test, $P<0.001$).

Pharmacodynamics

Correlation between busulfan disposition at steady state and HVOD could be investigated in 44 out of 45 patients. No difference was observed in terms of age, sex, clearance and AUC between the BU MEL group and the BU TTP group. However, an important factor that had to be taken into account was the fact that the BU MEL group had received more prior chemotherapy courses than the BU TTP group ($P<0.0001$). As a higher incidence of HVOD was observed in the BU MEL group as compared to the BU TTP group (not statistically significant), we decided to conduct two separate analyses.

There was no difference in terms of systemic exposure to busulfan between patients who developed HVOD and those who did not in the BU MEL group (Table 2,

Table 2 Pharmacodynamics after BU MEL

	No. of HVOD	HVOD	P
Number of evaluable patients	14	11	
Sex ratio (M/F)	7/7	8/3	NS
Age (years)	9.9 (2.1-19.4)	13.1 (1.2-20.0)	NS
Dose (mg/m^2)	604 ± 38	601 ± 10	NS
Number of lines/prior CT	2 (1-4)	2 (1-3)	NS
<i>Pharmacokinetics - dose 13</i>			
Number of evaluable patients	13	11	
AUC_{0-6h} ($h\ ng\ ml^{-1}$)	5318 ± 1145	4389 ± 1314	NS
CI/F ($ml\ min^{-1}\ m^{-2}$)	124 ± 27	163 ± 55	NS
C_{max} ($ng\ ml^{-1}$)	1340 ± 294	1283 ± 654	NS
<i>Trough levels ($ng\ ml^{-1}$)</i>			
Dose 1 - 1800 h	332 ± 123	331 ± 118	NS
Dose 2 - 0000 h	460 ± 140	434 ± 188	NS
Dose 3 - 0006 h	720 ± 209	507 ± 235	<0.05
Dose 4 - 1200 h	589 ± 223	402 ± 181	NS
Dose 12 - 1200 h	550 ± 242	420 ± 176	NS
Dose 13 - 1800 h	477 ± 163	375 ± 130	NS

Results are means \pm s.d. or medians (ranges).

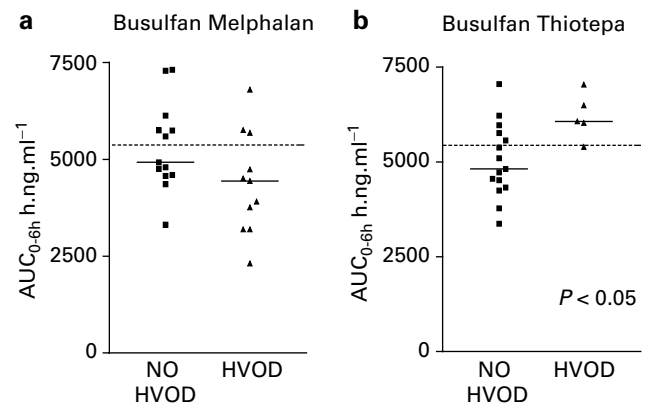


Figure 2 Systemic exposure to busulfan in relation with HVOD after BU MEL (a) or BU TTP (b). AUC_{0-6h} after the 13th dose of busulfan related to HVOD. Plots are representing two categories of patients: those with HVOD (\blacktriangle) and those without (\blacksquare); Slattery's threshold ($5400\ h\ ng\ ml^{-1}$) is represented by a dash line; medians are represented by a solid line.

Figure 2a). Moreover, a trend towards a lower mean AUC_{0-6h} was observed in patients who developed HVOD ($4389 \pm 1314\ h\ ng\ ml^{-1}$) as compared to patients in whom hepatotoxicity was not observed ($5318 \pm 1145\ h\ ng\ ml^{-1}$) ($P=0.06$). In addition, six out of 13 patients free of HVOD had an AUC_{0-6h} above the toxicity threshold defined by Slattery, as compared to only three out of 11 patients with HVOD. Moreover, all the mean busulfan trough levels in patients with HVOD were equal to or lower than those measured in patients free of HVOD (Table 2, Figure 2a). This difference was significant when trough plasma levels at 0600 h were considered (Table 2).

In contrast (Table 3, Figure 2b), patients who developed HVOD in the BU TTP group had a significantly higher AUC_{0-6h} than those who did not ($P<0.05$). Four of the five patients with HVOD had an AUC_{0-6h} above the $5400\ h\ ng\ ml^{-1}$ threshold. In addition, the fifth patient

Table 3 Pharmacodynamics after BU TTP

	No. of HVOD	HVOD	P
Number of evaluable patients	15	5	
Sex ratio (M/F)	10/5	2/3	NS
Age (years)	3.5 (1.8–15.7)	4.8 (1.1–13.5)	NS
Dose (mg m ⁻²)	601 ± 22	554 ± 91	NS
Number of lines/prior CT	1 (1–2)	1 (1–2)	NS
<i>Pharmacokinetics – dose 13</i>			
Number of evaluable patients	15	5	
AUC _{0–6h} (h ng ml ⁻¹)	5024 ± 978	6201 ± 607	<0.05
Cl/F (ml min ⁻¹ m ⁻²)	129 ± 25	93 ± 12	<0.01
C _{max} (ng ml ⁻¹)	1377 ± 295	1581 ± 214	NS
<i>Trough levels (ng ml⁻¹)</i>			
Dose 1 – 1800 h	347 ± 179	372 ± 136	NS
Dose 2 – 0000 h	398 ± 241	511 ± 111	NS
Dose 3 – 0006 h	554 ± 297	587 ± 244	NS
Dose 4 – 1200 h	406 ± 238	716 ± 186	<0.05
Dose 12 – 1200 h	448 ± 196	617 ± 143	NS
Dose 13 – 1800 h	353 ± 99	447 ± 141	NS

Results are means ± s.d. or medians (ranges). AUC_{0–6h}, area-under-the-curve at steady state after the 13th dose; CT, conventional chemotherapy; Cl/F, total clearance (Cl) uncorrected for bioavailability (F); C_{max}, maximal concentration of busulfan assayed in plasma samples during the 13th dose.

with HVOD had an AUC_{0–6h} attaining 5392 ng h ml⁻¹ which was very close to the threshold. Moreover, mean trough levels in patients with HVOD were always higher than those observed in patients free of HVOD. This statistically significant difference was observed at 2400 h on day 1, that is, after the fourth dose (*P* < 0.05, Table 3).

Discussion

Busulfan disposition after oral administration has been extensively studied in both adults and children.¹⁴ Busulfan pharmacokinetics have been demonstrated to be highly variable and many teams have been searching for a correlation between systemic exposure to busulfan and toxicity or failure to engraftment.^{13,15,17,22,28,30–32}

Slattery *et al*¹⁶ defined a therapeutic window for adult patients treated with BU CY and undergoing allogeneic HSCT to ensure BM engraftment (3600 h ng ml⁻¹, minimum threshold for efficacy) and to avoid severe regimen-related toxicity (5400 h ng ml⁻¹, maximum threshold for toxicity). In that study, severe regimen-related toxicity (grade 3/4) was encountered in one out of 31 patients (3%, CI_{95%} [1–17%]) below 5400 h ng ml⁻¹ and in four out of 11 patients (36%, CI_{95%} [11–69%]) in whom the AUC was above 5400 h ng ml⁻¹. Similar results have been obtained elsewhere^{13,15,30} and therefore prospective pharmacokinetically guided dose adjustment is currently performed for adult patients treated with BU CY.³³

The minimum threshold 3600 h ng ml⁻¹ has been demonstrated to ensure engraftment in children undergoing allogeneic stem cell transplantation after a conditioning BU CY regimen. However, data are lacking to demonstrate a relationship between overexposure to busulfan and liver toxicity in children and to define a maximum threshold in

this population.^{17,34} The therapeutic window (3600–5400 h ng ml⁻¹) developed for adults has however been successfully applied in children^{19,21} under treatment with BU CY in whom therapeutic drug monitoring (TDM) was mainly aimed at ensuring engraftment with exposure to busulfan exceeding 3600 h ng ml⁻¹. The therapeutic window defined for BU CY cannot be used prospectively to adjust the dose of busulfan in children treated with BU MEL or BU TTP regimens. A clear PK/PD relationship must first be defined during treatment with these regimens. The aim of TDM during high-dose chemotherapy with autologous HSCT is to reach the maximal tolerated dose (MTD) in order to optimize the antitumor effect. Undue busulfan dose reduction during BU MEL or BU TTP may jeopardize treatment efficacy. We designed a prospective study to explore the relationship between exposure to busulfan and liver toxicity during BU MEL or BU TTP.

In the present series, we report a high incidence of HVOD (44%) after a BU MEL conditioning regimen. Our aim was to develop busulfan TDM in order to reduce this unacceptable HVOD incidence and the first step was to establish a PK/PD relationship and to design a therapeutic index. Unfortunately, the occurrence of HVOD was not correlated with high systemic exposure to busulfan, as evidenced by steady-state AUCs and trough levels. These results are at variance with those reported in adults after a BU CY regimen because patient exposure to drugs was similar (up to 8220 h ng ml⁻¹).¹⁶ It has been demonstrated that for busulfan total dose of 600 mg m⁻², the exposure of pediatric patients was equivalent to the exposure of adult patients receiving 16 mg kg⁻¹.²⁵ High-dose melphalan may therefore be an independent risk factor for HVOD, and high systemic exposure to melphalan may be associated with a high risk of liver toxicity. We recently showed that hematopoietic and gastrointestinal toxicity were correlated with systemic exposure to melphalan in children receiving high-dose melphalan as a single agent.³⁵ However, no liver toxicity was observed when melphalan was used alone. Furthermore, HVOD has not been reported during high-dose chemotherapy using melphalan at a dose attaining 200 mg m⁻² in adult patients treated for multiple myeloma.³⁶ Since single-agent melphalan is not known to be hepatotoxic, a drug interaction between busulfan and melphalan warrants investigation to explain the occurrence and incidence of hepatotoxicity. Both busulfan^{37,38} and melphalan³⁹ are metabolized *in vivo* via glutathione (GSH)-conjugation by glutathione-S-transferases (GSTs). The GSH/GSTs pathway plays a key role in melphalan detoxification and drug resistance.^{40,41} *l*-Buthionine-[*S,R*]-sulfoximide (BSO), a γ -glutamylcysteine synthetase inhibitor, gradually depletes the glutathione intracellular pool thereby increasing melphalan cytotoxicity against many cell lines, human neuroblastoma cell lines being a case in point. Moreover, Shulman *et al*⁴² showed that dogs develop a similar form of HVOD to that observed in humans when melphalan was combined with busulfan. This animal model suggested that GSH depletion sensitizes the liver to melphalan. Busulfan has also been demonstrated to deplete GSH cellular levels *in vitro*.⁴³ Taken together, these data suggest that exposure to busulfan during treatment with a high-dose BU MEL regimen induces hepatic GSH

depletion that sensitizes the liver to the toxic effects of melphalan. This hypothesis is supported by our previously reported findings following an investigation of risk factors for HVOD in 136 children receiving a high-dose busulfan-containing regimen.¹² The multivariate analysis in that study showed that the position of busulfan in regimens combining three alkylating agents, namely busulfan, cyclophosphamide and either melphalan or thiotepea, was an independent risk factor for HVOD. Indeed, the relative risk was 3.59 when busulfan was the first drug administered as compared to 1.27 when busulfan was in second place, that is, after melphalan.¹² Hassan *et al*⁴⁴ recently reported a possible drug interaction between busulfan and cyclophosphamide via GSH/GSTs and cytochromes P450 pathways.⁴⁴ We postulate that the absence of a relationship between exposure to busulfan and liver toxicity during treatment with BU MEL is likely due to a drug interaction that augments melphalan-induced liver toxicity as a result of prior busulfan-induced GSH depletion.

Conversely, exposure to busulfan was shown to be significantly higher after a BU TTP regimen in patients who developed HVOD. However, these data do not suggest that the toxicity threshold defined by Slattery *et al*¹⁶ in patients treated with a BU CY conditioning regimen might be relevant for the prospective adjustment of the busulfan dose in children conditioned with BU TTP. Indeed, it is noteworthy that systemic exposure to busulfan was above this toxicity threshold in five of the 15 patients who did not develop HVOD. An AUC below 5400 h ng ml⁻¹ apparently limits the incidence of HVOD but there are two categories of patients above this threshold, those who develop HVOD and those who do not. How can these two categories be explained? We support the hypothesis proposed by Deleve and Wang⁴³ to explain busulfan-induced toxicity in hepatocytes with a normal GSH level. Under such conditions busulfan-induced liver toxicity may be due to the formation of busulfan sulfonium conjugate, a compound that promotes toxic oxidative stress. Thus, patients with a busulfan AUC above the 5400 h ng ml⁻¹ toxicity threshold may therefore develop HVOD only when the balance between the production of oxidative stress and cellular defenses is disrupted. We hypothesize that this disruption could be due to wide differences in focal production of busulfan sulfonium conjugate in the liver, because this reaction is catalyzed mainly by GSTA1-1 and GSTM1-1, enzymes whose expression in tissue is highly variable and which exhibit well-documented genetic polymorphisms.^{45,46}

As far as drug monitoring is considered, accurate limited sampling strategies should be developed, especially in children. This is why we have established a limited sampling model (LSM) using a stepwise regression model at an early stage in our busulfan pharmacology program. Several other teams have reported different LSMs.⁴⁷ The present study was also designed to evaluate the accuracy of our LSM model in estimating systemic exposure to busulfan after the first dose. The estimation predicted by this model was poor, probably because of the wide interpatient variability in busulfan absorption. Other approaches using Bayesian modeling, such as that recently reported by Sandstrom *et al*,¹⁸ should be preferred.

To conclude, we have demonstrated in our experience, the absence of a relationship between exposure to busulfan and liver toxicity during BU MEL. We postulate that this phenomenon is due to a drug interaction between busulfan and melphalan via the GSTs/GSH pathway. So there is no rationale to use pharmacokinetically guided dose adjustment of busulfan during BU MEL regimen. In contrast, patients in the BU TTP group who developed HVOD had a significantly higher exposure than those free of HVOD. This observation, obtained with a low number of patients, has to be confirmed before to evaluate prospectively therapeutic drug monitoring of busulfan during BU TTP. To use an i.v. formulation of busulfan and to reduce variability due to oral ingestion^{48,49} would be useful to refine these data.

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