

Influence of prophylactic anticonvulsant therapy on high-dose busulphan kinetics

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Abstract. The pharmacokinetics of high-dose busulphan was studied in 17 patients during conditioning prior to bone marrow transplantation using deuterium-labeled busulphan (d_3 -BU). About 50% of busulphan doses 1 and 16 was replaced with d_3 -BU. Patients were treated with phenytoin or diazepam as prophylactic anticonvulsant therapy. Patients who received phenytoin demonstrated significantly higher clearance (mean \pm SD, 3.32 ± 0.99 ml min⁻¹ kg⁻¹), a lower area under the concentration-time curve (AUC, $5,412 \pm 1,534$ ng h ml⁻¹; corrected for dose/kilogram) and a shorter elimination half-life (3.03 ± 0.57 h) for the last dose of d_3 -BU (dose 16) as compared with the first dose (2.80 ± 0.78 ml min⁻¹ kg⁻¹, $6,475 \pm 2,223$ ng h ml⁻¹ and 3.94 ± 1.10 h, respectively). No difference in the above-mentioned pharmacokinetic parameters was seen in patients treated with diazepam. Moreover, a continuous decrease in the steady-state level of busulphan was observed in four of seven patients in the phenytoin-treated group, whereas in the diazepam group, such a decrease was seen in only one of eight patients. We conclude that phenytoin used as prophylactic anticonvulsant therapy alters busulphan pharmacokinetics and, most probably, its pharmacodynamics. For adequate prophylactic therapy, anticonvulsants with fewer enzyme-inductive properties than phenytoin should be used.

Introduction

During the last decade, high-dose chemotherapy with or without total body irradiation followed by allogeneic (BMT) or autologous bone marrow transplantation (ABMT) has been increasingly used as a very effective means of treating both haematological malignancies and inborn errors of metabolism [1, 2]. High-dose busulphan (HD-BU) in combination with cyclophosphamide was introduced by Santos et al. [3] as a myeloablative regimen for bone marrow transplantation. Studies of HD-BU in both adults and children [4–6] have shown that patients reach a steady-state level after the third dose and that busulphan has age-related pharmacokinetics. Veno-occlusive disease, mucositis, interstitial pneumonitis and haemorrhagic cystitis are well-known side effects related to HD-BU therapy [7, 8]. HD-BU therapy is also associated with seizures [9–12], and its dose-dependent neurotoxicity has been studied in children [13]. The convulsions may be a result of busulphan's crossing the blood brain barrier rapidly [14] and entering the cerebrospinal fluid compartment at a concentration of the same magnitude as that found in plasma [5, 15].

Many transplantation protocols include the anticonvulsants phenytoin and diazepam as routine prophylaxis to avoid seizures. Several antiepileptic agents, including phenytoin, phenobarbital and valproate, are known to induce liver enzymes such as cytochrome P450 and γ -glutamyl transferase (γ -GT) [16–18]. In a murine model, Fitzsimmons et al. [19, 20] have shown that phenytoin administration decreases the myelotoxicity and the neurotoxicity of busulphan and leads to higher survival of normal mice. Moreover, we showed in a recent study a continuous decrease in the steady-state levels of busulphan in about 40% of patients (both adults and children) over the 4-day therapy period [21].

Busulphan is extensively metabolized in the rat liver, mostly via an enzymatic reaction with glutathione [22, 23]. It has also been shown that busulphan is at least partly metabolized via the glutathione route in humans [4]. An activation of the hepatic enzymes would enhance the

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Abbreviations: AML, acute myelocytic leukaemia; ALL, acute lymphocytic leukaemia; MDS, myelodysplastic syndrome; ABMT, autologous bone marrow transplantation; BMT, allogeneic bone marrow transplantation

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Table 1. Clinical data for patients treated with phenytoin as an anticonvulsant: group A

Patient number	Age (years)	Sex	Weight (kg)	Busulphan dose (mg)	Doses 1 and 16 of d ₅ -BU (mg)	Diagnosis	Transplantation type
1	54	M	78	75	40	Lymphoma	BMT
2	58	M	78	80	30	AML	ABMT
3	53	M	94	95	50	AML	ABMT
4	51	M	68	68	30	AML	ABMT
5	52	F	53	54	30	AML	ABMT
6	43	M	80	81	40	AML	ABMT
7	40	F	56	60	30	CML	BMT
8	38	M	83	81	40	ALL	ABMT
9	60	M	70	70	30	AML	ABMT

AML, Acute myeloblastic leukaemia; CML, chronic myelocytic leukaemia; ALL, acute lymphocytic leukaemia

Table 2. Clinical data for patients treated with diazepam as an anticonvulsant: group B

Patient number	Age (years)	Sex	Weight (kg)	Busulphan dose (mg)	Doses 1 and 16 of d ₅ -BU (mg)	Diagnosis	Transplantation type
1	33	F	88	100	50	Lymphoma	BMT
2	30	M	63	64	30	MDS	ABMT
3	56	M	65	66	30	AML	ABMT
4	40	M	64	75	40	AML	ABMT
5	19	M	61	60	30	AML	ABMT
6	47	M	66	63	30	AML	ABMT
7	25	M	91	91	40	CML	BMT
8	45	M	84	85	40	CML	BMT

MDS, Myelodysplastic syndrome; AML, acute myeloblastic; CML, chronic myelocytic leukaemia

metabolism of busulphan, resulting in less effective therapy.

In the present study we investigated the effect of the anticonvulsants phenytoin and diazepam on busulphan kinetics during high-dose treatment. The study was performed by the administration of deuterium-labeled busulphan (d₅-BU) to patients undergoing BMT/ABMT as 50% of both dose 1 and dose 16. We report on an interaction between phenytoin and busulphan that resulted in a significant difference in the pharmacokinetics of busulphan between the first and the last dose. Also, a continuous decrease in the steady-state level during HD-BU therapy was found in some patients.

Patients and methods

Chemistry. d₅-BU was synthesized from [1,1,2,2,3,3,4,4-d₅]-1,4-butanediol (d₅-butanediol) obtained from Aldrich (Germany). Recrystallized methanesulphonic anhydride (1 mol; Merck, Germany) was dissolved in dichloromethane (800 ml; Merck) and dropped slowly into a solution of d₅-butanediol (0.1 M in 50 ml pyridine; Merck, Germany) and placed in an ice/NaCl bath. The temperature was not allowed to exceed 4°C and the solution was stirred overnight. The reaction mixture was washed twice with 100 ml sulphuric acid (1 M) and the dichloromethane was evaporated to dryness. The residue was redissolved in acetone and then precipitated with n-hexane. The yield of d₅-BU (22.2 g) was 90%. The melting point was 115°C (reported for busulphan 115°–117°C). d₅-BU was identified by nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS). The NMR analysis was performed on a Varian 300-MHz apparatus and the analysis of both busulphan

and d₅-BU showed the following shifts: a singlet at 3.120 ppm (CH₂-) for d₅-BU as compared with a singlet at 3.120 ppm (CH₂-), a triplet at 4.326 ppm (O-CH₂-) and a triplet at 1.906 ppm (CH₂-CH₂) for busulphan.

After conversion of busulphan and d₅-BU to the corresponding 1,4-diiodobutane [14], GC-MS was performed using a 10-m × 0.57-mm CB-sil 8 column (Chrompack, Holland) with temperature programming (90°–180°C). Injector and ion-source (70 eV) temperatures were 250°C and the separator temperature was 270°C. The identification of d₅-BU was based on the ions' m/z values [318 (7%), 191 (100%), 159 (32%), and 62 (60%)], which compared with the ions obtained from busulphan's m/z values [310 (12%), 183 (93%), 155 (73%), and 55 (100%)].

d₅-BU was tested for bacterial contamination and was found to be sterile. The labeled busulphan was weighed under sterile conditions and encapsulated in gelatine capsules of 10 mg each. The study was approved by the local ethics committee.

Patients. A total of 17 patients with either leukaemia or Hodgkin's disease were prepared for either BMT or ABMT. The clinical data for the two groups of patients are listed in Tables 1 and 2, respectively. Nine patients (group A, Table 1) received phenytoin as a prophylaxis anticonvulsant, starting with a loading dose of 5 mg/kg × 4 given from day -10 to day -8, followed by 2.5 mg/kg × 2 given from day -7 to day -4. The second group (group B, Table 2) was treated with diazepam given at 5 mg p.o. × 4 from day -7 to day -4.

The preparatory chemotherapy regimen for both groups consisted of HD-BU given p.o. at 1 mg/kg × 4 for 4 days starting on day -7, as shown in Tables 1 and 2, followed by cyclophosphamide given i.v. at 60 mg/kg for 2 days. In all patients, about 50% of busulphan doses 1 and 16 (Fig. 1) was replaced by d₅-BU capsules, and the treatment was started at 8:00 a.m. to minimize the risk of chronopharmacological effects as described previously [21, 24]. The doses of busulphan and d₅-BU are shown in Tables 1 and 2.

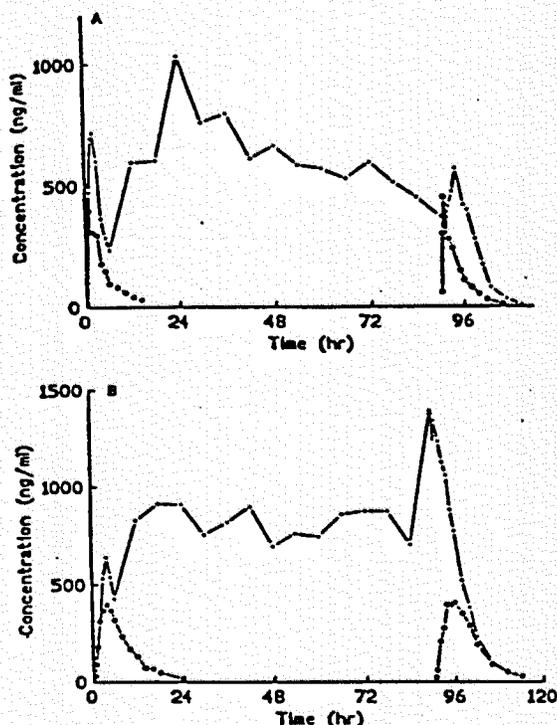


Fig. 1. A Plasma concentrations of busulphan and d_4 -BU measured in patient 9 of the phenytoin-treated group, showing a decrease in steady-state levels during the treatment period. - - -, Busulphan; ●—●, d_4 -BU during the first and last doses. B Plasma concentrations of busulphan and d_4 -BU measured in patient 4 of the diazepam-treated group, showing no decrease in steady-state levels during the treatment period. - - -, Busulphan; ●—●, d_4 -BU during the first and last doses

Sample collection. Blood samples (4–7 ml) were drawn from a central venous catheter into heparinized glass tubes. The sampling was more frequent during the first and the last dose (16–20 samples) as compared with the other doses. For determination of the mean minimal concentration during the 4 days of treatment, one blood sample was drawn immediately before each dose interval. The plasma was separated by centrifugation and stored at -20°C until analysis.

Busulphan and d_4 -BU determination. Internal standard (IS) [1,5-bis-(methanesulfonyl)pentane], sodium iodide and *n*-heptane were added to the plasma samples (1 ml). Busulphan, d_4 -BU and IS were determined after their conversion to 1,4-diiodobutane, 1,4- d_4 -diiodobutane and 1,5-diiodopentane, respectively, either by GC with electron-capture detection [25] or with GC-MS in the selected ion-monitoring mode (SIM) [26].

Pharmacokinetic analysis. Pharmacokinetic parameters for d_4 -BU and for the last dose of busulphan were calculated using the PCNONLIN program (Statistical Consultants, Inc., USA). The plasma-concentration data were analysed, and values for the maximal plasma concentration (C_{max}), the time required to reach C_{max} (t_{max}), the area under the plasma concentration-time curve (AUC) and the plasma elimination half-life ($t_{1/2}$) for the first and the last dose of d_4 -BU and the last dose of busulphan were calculated according to a linear one-compartment open model. The apparent terminal slopes following only the first dose of busulphan were calculated by semilogarithmic regression analysis. The differences within the groups were established using Student's paired *t*-test, whereas differences between the groups were analysed using Student's unpaired *t*-test and two-sample analysis. The decrease in steady-state level for each patient was established using linear regression analysis of the mean minimal concentrations determined (the concentration of busulphan measured immediately before each dose) after the steady-state level had been reached (doses 4–16).

Results

The pharmacokinetic parameters for both dose 1 and dose 16 (d_4 -BU) are given in Table 3 for the group of patients who received phenytoin as anticonvulsant prophylaxis (group A) and in Table 4 for those who received diazepam (group B).

Table 3. Pharmacokinetic parameters for d_4 -BU in phenytoin-treated patients

Patient number	First dose						Last dose					
	C_{max} (ng/ml)	t_{max} (h)	AUC (ng h ml $^{-1}$)	Cl (ml min $^{-1}$ kg $^{-1}$)	Vd (l kg $^{-1}$)	$t_{1/2}$ (h)	C_{max} (ng/ml)	t_{max} (h)	AUC (ng h ml $^{-1}$)	Cl (ml min $^{-1}$ kg $^{-1}$)	Vd (l kg $^{-1}$)	$t_{1/2}$ (h)
1	412	2.61	6,025	2.77	0.82	3.12	418	2.49	5,831	2.86	0.77	3.06
2	530	1.49	11,355	1.47	0.83	6.49	344	1.95	7,216	2.31	0.82	4.03
3	368	2.44	5,255	3.17	0.99	3.74	394	2.12	4,345	3.84	0.85	2.57
4	533	3.31	4,793	3.48	0.93	2.72	276	2.18	4,052	4.11	0.84	2.33
5	614	1.34	8,104	2.05	0.42	3.69	693	1.88	7,657	2.18	0.34	2.81
6	476	2.44	7,446	2.24	0.76	3.94	616	0.96	6,554	2.54	0.76	3.41
7	467	1.65	4,498	3.71	1.16	3.60	333	1.24	3,255	5.12	1.07	2.52
8	427	0.49	6,231	2.67	1.08	4.68	411	1.50	5,565	3.01	0.95	3.67
9	466	0.77	4,572	3.65	1.08	3.48	381	0.86	4,233	3.94	0.96	2.83
Mean \pm SD	477 \pm 74	1.84 \pm 0.92	6,475 \pm 2,223	2.80 \pm 0.78	0.90 \pm 0.23	3.94 \pm 1.10	430 \pm 136	1.69 \pm 0.57	5,412 \pm 1,534	3.32 \pm 0.99	0.82 \pm 0.21	3.03 \pm 0.57

C_{max} , Maximal plasma concentration; t_{max} , time for C_{max} ; AUC, area under the plasma concentration-time curve (corrected for dose/kg); $t_{1/2}$, plasma elimination half-life; Vd, distribution volume; Cl, total body clearance based on $f = 1$

Table 4. Pharmacokinetic parameters for d_8 -BU in diazepam-treated patients

Patient number	First dose						Last dose					
	C_{max} (ng/ml)	t_{max} (h)	AUC (ng h ml ⁻¹)	Cl (ml min ⁻¹ kg ⁻¹)	Vd (l kg ⁻¹)	$t_{1/2}$ (h)	C_{max} (ng/ml)	t_{max} (h)	AUC (ng h ml ⁻¹)	Cl (ml min ⁻¹ kg ⁻¹)	Vd (l kg ⁻¹)	$t_{1/2}$ (h)
1	615	1.67	6,536	2.55	0.72	3.22	698	1.19	7,816	2.12	0.61	3.36
2	572	0.65	5,856	2.85	0.77	3.14	440	1.82	5,427	3.05	0.84	3.21
3	486	1.61	6,346	2.63	0.83	3.62	357	2.60	6,904	2.42	0.83	3.96
4	331	3.76	5,611	2.97	1.05	4.07	406	6.19	7,270	2.27	0.84	4.25
5	440	2.51	6,698	2.49	0.72	3.30	550	2.05	8,049	2.08	0.62	3.44
6	540	2.11	11,516	1.45	0.78	6.28	428	1.90	7,760	2.17	0.80	4.28
7	499	2.27	7,727	2.16	0.68	3.67	409	1.43	6,841	2.43	0.88	4.19
8	366	3.49	6,390	2.61	0.58	2.59	334	3.75	6,306	2.63	0.71	3.14
Mean	481 ± 98	2.26 ± 1.01	7,085 ± 1,898	2.46 ± 0.47	0.77 ± 0.14	3.74 ± 1.12	453 ± 118	2.62 ± 1.65	7,047 ± 877	2.40 ± 0.32	0.77 ± 0.11	3.73 ± 0.49

C_{max} , Maximal plasma concentration; t_{max} , time for C_{max} ; AUC, area under the plasma concentration-time curve (corrected for dose/kg); $t_{1/2}$, plasma elimination half-life; Vd, distribution volume; Cl, total body clearance based on $f = 1$.

The time required to reach the C_{max} of d_8 -BU in both groups varied between 0.49 and 6.19 h. No difference was observed between the first and the last dose within either group, whereas a slightly shorter t_{max} was found in group A as compared with group B for both doses. This difference was similar to that seen for busulphan at dose 16 between the groups (1.70 ± 1.38 h for group A vs 2.04 ± 1.65 h for group B). The absorption of d_8 -BU in group B for both the first and the last dose obeyed first-order kinetics, whereas in group A, seven of nine patients displayed a zero-order absorption mechanism.

The AUC (corrected for dose/kilogram) for the first dose of d_8 -BU was significantly higher (6,475 ± 2,223 ng h ml⁻¹; $P = 0.02$) than that for the last dose (5,412 ± 1,534 ng h ml⁻¹) in group A patients, whereas no difference between the first and the last dose was seen in group B in that respect. The AUC for the last dose was significantly lower in group A than in group B ($P = 0.01$). No difference was obtained when the AUCs for the first dose were compared between the two groups.

Patients treated with phenytoin showed a significantly higher ($P = 0.006$) clearance of d_8 -BU after the last dose (3.32 ± 0.99 ml min⁻¹ kg⁻¹) as compared with the first dose (2.80 ± 0.78 ml min⁻¹ kg⁻¹). The clearance of d_8 -BU after the last dose was also higher in group A as compared with group B ($P = 0.03$). Clearance did not change significantly from the first to the last dose of d_8 -BU in patients of group B (2.46 and 2.40 ml min⁻¹ kg⁻¹, respectively). Moreover, busulphan's clearance (last dose) showed a tendency to be higher in group A than in group B (3.30 ± 0.44 vs 2.85 ± 0.47 ml min⁻¹ kg⁻¹).

The elimination of d_8 -BU was significantly faster ($P = 0.003$) after the last dose (3.03 ± 0.57 h) as compared with the first dose (3.94 ± 1.10 h) in group A, whereas no difference was observed in group B ($t_{1/2}$ 3.73 and 3.74 h, respectively). There was a significant difference ($P = 0.01$) in the last-dose elimination half-lives observed between group A and group B.

The elimination half-life determined for the last dose of busulphan in group A was slightly ($P = 0.06$) shorter (2.70 ± 0.83 h; range, 1.39–4.24 h) than that found for

Table 5. Decrease in steady-state levels of busulphan for both patient groups

Patient number (group)	Intercept (ng/ml)	Slope	Significance level (P)	Decrease after 16 doses (%)
6 (A)	1,540	-14.81	0.0005	52
7 (A)	637	-3.51	0.005	22
8 (A)	1,467	-9.29	0.0001	32
9 (A)	1,092	-8.01	0.002	27
6 (B)	1,438	-8.18	0.02	20

the first dose (3.59 ± 1.29 h; range, 0.94–5.13 h; the $t_{1/2}$ for the first dose was calculated for each patient on the basis of 3–4 observations). However, in group B the elimination half-life determined for the last dose (2.98 ± 0.33 h; range, 2.40–3.40 h) did not differ significantly ($P = 0.2$) from that calculated for the first dose (3.38 ± 1.14 h; range, 2.34–5.81 h).

No change in the distribution volume of d_8 -BU was observed during busulphan conditioning. Distribution volumes of 0.90 ± 0.23 and 0.82 ± 0.21 l kg⁻¹ were obtained in group A during the first dose and the last dose, respectively. For group B, distribution volumes of 0.77 ± 0.14 l kg⁻¹ for the first dose and 0.77 ± 0.11 l kg⁻¹ for the last dose were calculated. These values were similar to those obtained for groups A and B during the last dose of busulphan (0.64 ± 0.18 and 0.73 ± 0.10 l kg⁻¹, respectively).

A continuous and significant decrease in the steady-state level of busulphan was found in four of seven patients in group A (Table 5). The percentage of decrease was calculated from the regression line as the ratio between the plasma level measured at dose 16 and that determined at dose 3. On the other hand, only one individual (patient 6) in group B showed a continuous decrease by 20%. This patient displayed an extremely long elimination half-life for both busulphan and d_8 -BU during the first and last doses.

Discussion

Busulphan has been used in conditioning regimens prior to bone marrow transplantation more frequently during the last decade. Busulphan is a lipophilic small compound that is rapidly absorbed from the gastrointestinal tract and highly metabolized in the liver. In the present study, deuteration of busulphan did not result in a significant isotope effect. Only a slightly longer plasma elimination half-life was observed, which might have been due to an increase in busulphan's lipophilicity or to a slower enzymatic reaction of the deuterated analogue. However, this difference in plasma half-life was of the same magnitude for both the first and the last dose and was seen in both patient groups, which most likely makes both busulphan and *ds*-BU pharmacokinetically comparable.

The dose-dependent neurotoxicity of busulphan is well established [13], and many cases of convulsions have been reported, mostly in adults. Martell et al. [9] reported a myoclonic case of epilepsy; Marcus and Goldman [11] described a case of consciousness loss and intermittent muscle twitching; and De La Camara et al. [12] described three cases of generalized seizures that did not result in neurological deficits which occurred despite the use of anticonvulsant prophylaxis in two of these three patients. Santos [27] estimated the rate of occurrence of convulsions to be 10% in over 100 adult patients with acute leukaemia. The rate of convulsions was reported to be 1.7% in very young children receiving 16 mg/kg busulphan and 15.4% in young children when the dose was adjusted to 600 mg/m². This difference was due to higher systemic exposure as reported by Vassal et al. in a recent study [13].

However, convulsions in adults have been described mostly as generalized tonic-clonic convulsions without neurological sequelae, usually occur on the 2nd day of treatment [10, 11] and can even occur at 18–24 h after the last dose as reported by Grigg et al. [28]. A possible reason for these convulsions might be the continuous exposure of the brain to at least 20% of the busulphan dose during 4-day treatment [14, 15] or an accumulation of busulphan metabolites in the brain, which has been noted in the rat brain [29].

To prevent the occurrence of seizures, clonazepam was recommended by Vassal et al. [13] as prophylaxis for children, whereas phenytoin has been recommended by Sureda et al. [10] and Grigg et al. [28] for adult patients. Phenytoin is a common, widely used anticonvulsant agent. It has been shown that phenytoin induces serum bilirubin [30] and many liver enzymes such as γ -glutamyltransferase (γ -GT) in adult epileptic patients [31], and in children it induces γ -GT and may cause liver damage [17].

In the present investigation, phenytoin-treated patients displayed significantly shorter elimination half-lives, higher clearance and lower AUCs after the last dose of *ds*-BU as compared with the first dose. On the other hand, none of the above-mentioned parameters was affected when diazepam was used as prophylaxis in seven of eight patients. Also, none of the patients in either group showed any sign of neurotoxicity during HD-BU therapy. A continuous decrease in the steady state-level of busulphan was observed in four of seven patients in the phenytoin-treated

group as compared with only one of the patients receiving diazepam. These results are in good agreement with those of our previous study [21], in which we reported that about 40% of the patients showed a continuous decrease in the steady-state level of busulphan. In a retrospective analysis, it was found that about 90% of those patients were treated with phenytoin as anticonvulsant prophylaxis.

The present results might explain the observations reported by Fitzsimmons et al. [19, 20], who were capable of decreasing the neurotoxicity and myelotoxicity of busulphan in a murine model pretreated with phenytoin. In this respect, busulphan does not differ from many other drugs such as misonidazole, prednisolone and paracetamol, for which it has been shown that phenytoin administration can alter their pharmacokinetics, pharmacodynamics and toxicity [32–35].

On the other hand, Grigg et al. [28] reported that three patients experienced myoclonic jerks during HD-BU therapy given in combination with phenytoin as an anticonvulsant. In two of these cases the phenytoin levels were about 10 mmol/l, which is far from the therapeutic range (40–80 mmol/l). Many authors have reported decreased phenytoin levels during antineoplastic therapy with a combination regimen of carmustine, methotrexate and vinblastine or cisplatin, vinblastine and bleomycin [36–38]. However, the levels of phenytoin were normalized after termination of the antineoplastic therapy. In all three of the above-cited publications it was concluded that malabsorption was the most likely explanation for the decreased phenytoin levels, whereas an increase in the distribution volume or an increase in the rate of metabolism of phenytoin during antineoplastic therapy was given as an explanation by Neef and De Voogd-van der Straaten [39]. Since it is known that busulphan is extensively metabolized in both the rat and the human liver [4, 22, 23] and that phenytoin therapy can enhance liver activity, it is obvious that phenytoin administration can alter busulphan's pharmacokinetics and, most probably, its pharmacodynamics. In addition, it seems that busulphan, like other antineoplastic drugs [28, 35–38], can alter phenytoin's pharmacokinetics by inducing either malabsorption, a change in its distribution volume and/or an increase in the rate of its metabolism.

In summary, we conclude that phenytoin as an anticonvulsant alters the pharmacokinetics of busulphan and decreases its steady-state level during high-dose therapy, which most likely changes its pharmacodynamics, resulting in less effective myeloablative therapy prior to bone marrow transplantation. For adequate antiseizure prophylaxis, anticonvulsants with fewer enzyme-inductive properties than phenytoin should be used.

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Itraconazole Can Increase Systemic Exposure to Busulfan in Patients Given Bone Marrow Transplantation

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Abstract. Busulfan (BU) is an alkylating drug frequently used to prepare patients for bone marrow transplantation (BMT). Several studies have documented that there is important interpatient variability in BU disposition and systemic exposure, and that other drugs with a common metabolic pathway are capable of influencing BU clearance. We compared the BU pharmacokinetics and pharmacodynamics of 13 patients given BMT and receiving BU and itraconazole, with those of 26 matched controls who did not receive any anti-fungal agent, and with those of 13 matched patients treated with fluconazole as prophylaxis against fungal infections. The effect of itraconazole was best reflected in BU clearance since the BU dose was modified in some patients. BU clearance was decreased by an average of 20% in patients receiving itraconazole as compared to control patients and patients receiving fluconazole ($p < 0.01$). Mean BU clearance was $7.653 \pm 1.871 \text{ l/hr.m}^2$ in the itraconazole patients, $10.103 \pm 2.007 \text{ l/hr.m}^2$ in the fluconazole group and $9.373 \pm 1.702 \text{ l/hr.m}^2$ in the control group. In this study itraconazole, but not fluconazole, markedly affected the pharmacokinetics of BU as an increase of BU plasma

concentrations was observed. The nature of this interaction has not yet been fully characterized. Itraconazole and its analogues are inhibitors of both cytochrome P450 and lipoygenase and since itraconazole can modulate BU pharmacokinetics, oxidative catabolism is probably a determinant of BU metabolism. This hypothesis should be tested in human metabolic studies.

Due to its ability to permanently impair the self-renewal capacity of hematopoietic progenitors, busulfan (BU), usually associated to cyclophosphamide (Cy), has gained increasing use as an alternative to preparative regimens containing total body irradiation in patients given bone marrow transplantation (BMT) for both malignant and non-malignant disorders (1-4). The usual dose of BU employed is 1 mg/Kg every 6 hours for 16 doses and the pharmacokinetic parameters of the drug have been derived mostly from patients receiving this dosage. Several studies have documented that significant differences in the systemic exposure to the drug exist among patients, age representing the most important factor influencing BU disposition (5-8). In fact, in younger patients (particularly those below the age of 5) BU clearance and the volume of distribution have been reported to be higher than in adults and older children (9-12). In addition, since BU is mainly metabolized in the liver (13-18), several drugs can substantially modify its disposition, increasing the risk of either extra-haematological toxicities or inefficacy (19-20). Hepatic veno-occlusive disease (HVOD) is the most frequent life-threatening complication observed after

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Key Words: Busulfan, pharmacokinetics, bone marrow transplantation, conditioning regimen, antifungal treatment, itraconazole, drug-drug interaction.

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Table 1. Characteristics of the patients enrolled in the study.

	Itraconazole (n = 13)	Control Group (n = 26)	Fluconazole (n = 13)	P value
Median age (yrs. range)	22 (1-57)	26 (1-48)	26 (2-52)	P = NS
Males/Females	6/7	17/9	8/5	P = NS
Median body weight (kg. range)	53 (10-79)	52 (10-83)	53 (9-81)	P = NS
Median body surface area (m ² . range)	1.6 (0.42-1.90)	1.6 (0.43-2.1)	1.5 (0.38-2.1)	P = NS
Diseases:				
AML	4	8	4	P = NS
CML	6	12	6	
ALL	1	2	1	
Inborn Errors	2	4	2	
Conditioning regimens:				
BU/Cy 120	2	7	4	P = NS
BU/Cy 200	4	3	2	
BU/Cy-L-PAM	5	8	3	
BU/VP-16/Cy	2	8	4	
Type of BMT				
Autologous	3	2	3	P = NS
Allogeneic	10	24	8	

ALL = acute lymphoblastic leukemia; AML acute myelogenous leukemia; CML = chronic myelogenous leukemia; BU = busulfan; Cy = cyclophosphamide; L-PAM = melphalan; VP-16 = etoposide. *For further details on conditioning regimens see text. "Patients and Methods" section.

high-dose BU (21-23). Increased liver toxicity may be related to drug interaction in some conditioning regimens(24).

In BMT patients, particularly during the period of neutropenia, fungal infections represent one of the major causes of infectious death and the most difficult of all microbial infections to treat. Therefore, several studies have focused on the prophylactic use of systemic anti-fungal imidazoles such as itraconazole or fluconazole to prevent the occurrence of fungal infections. However, important drug interactions may occur resulting in either subtherapeutic or toxic plasma/tissue concentrations of different agents administered simultaneously.

We investigated whether the simultaneous administration of itraconazole can influence BU metabolism and consequently systemic exposure in comparison to patients who did not receive any kind of anti-fungal treatment.

Patients and Methods

Patients. Between January 1992 and June 1994, 13 consecutive patients (6 males and 7 females, age range 1-57 years) given an autologous (3 patients) or allogeneic marrow transplant (10 patients) and receiving BU and itraconazole, were enrolled in this prospective study. Written informed consent was obtained from patients or their parents. The control group included 26 patients, matched for body surface area, body weight, age and disease, who were given BMT in the period between January 1989 and April 1993 and did not receive any prophylaxis against fungal infections. Two controls were selected and assigned to each itraconazole-treated patient, thus resulting in a case:control ratio of 1:2.

Moreover, as fluconazole can be expected to influence BU metabolism, we also evaluated the impact of its administration on 13 further patients, matched with the previous 2 groups.

Considering the whole study population 16 patients were affected by acute myeloid leukemia (AML), 24 by chronic myeloid leukemia (CML), 4 by acute lymphoblastic leukemia (ALL) and 8 by inborn errors of the hematopoietic or immune system. Details on patients' characteristics and distribution among groups are reported in Table 1.

In the 3 groups of patients regimen-related toxicity was graded according to the criteria previously reported by Bearman *et al* (19).

Conditioning regimen. BU was given as whole or crushed tablets at a dose of 1 mg/kg every 6 hours on each of 4 consecutive days. A normal diet was offered on each day of BU administration. The conditioning regimens employed were as follows: a) BU/Cy 120: BU 4 mg/kg/day given orally from day -7 to day -4 and cyclophosphamide 60 mg/kg/day i.v. on days -3 and -2; b) BU/Cy 200: BU 4 mg/kg/day given orally from day -9 to day -6 and cyclophosphamide 50 mg/kg/day i.v. from day -5 to day -2; c) BU/Cy-L-PAM: BU 4 mg/kg/day given orally from day -7 to day -4, cyclophosphamide 60 mg/kg/day i.v. on days -3 and -2 and melphalan 140 mg/m² on day -1; d) BU/VP-16/Cy: BU 4 mg/kg/day given orally from day -7 to day -4, VP-16 20 mg/kg continuous infusion from day -6 to day -4 and cyclophosphamide 60 mg/kg/day i.v. on days -3 and -2.

Anti-fungal treatment. Itraconazole was administered orally at a single daily dose of 6 mg/kg starting from the day before the beginning of the conditioning regimen and ending at the time of myeloid recovery after BMT (absolute granulocyte count > 1 x 10⁹/L) or whenever the patient was unable to assume oral therapy or developed fungal infections requiring more aggressive treatment (i.e. amphotericin B intravenously). Likewise, fluconazole was administered orally at the same dosage starting from the day before the beginning of conditioning regimen and

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ending at time of myeloid recovery after BMT or whenever the patient was unable to assume oral therapy. In the latter case, some patients presenting grade 1 to 2 mucositis were shifted to the intravenous administration of fluconazole, whereas others were treated with amphotericin B intravenously.

Sample collection. Heparinized whole blood samples (3 ml) were obtained from indwelling venous catheters before and 0.5, 1, 2, 4, 6, 8 and 12 hours after the last dose of BU. Plasma was separated, frozen at -20 °C and shipped frozen until analysis.

Busulfan analysis. BU plasma levels were measured by an High Performance Liquid Chromatographic (HPLC) assay previously described by Henner(25). Plasma samples (0.3 ml) were mixed with 0.6 ml of methanol for deproteinization. The mixture was centrifuged to obtain the supernatant. Then, the supernatant was mixed with a 5% aqueous solution of diethylthiocarbamate (0.15 ml) and 100 mM ammonium acetate (pH 5.5). Immediately after combining the reagents, the derivative was extracted into ethyl acetate (1.5 ml) and dried in a speed vac concentrator Savant. HPLC was performed utilizing a column Supelcosil LC-18, 5 µ, 25 cm x 4.6 mm. The mobile phase consisted of 84% methanol and 16% water. The flow rate was 1.3 ml/min. The UV detector was at 251 nm.

The sensitivity limit of the assay was 20 ng/ml, and the intra- and interassay coefficients of variation were less than 8% over the entire range of the standard curve.

Pharmacokinetic analysis. Pharmacokinetic analysis was performed with P-PHARM computer software (version 1.1) (26). The individual pharmacokinetic parameters were assumed to arise from a normal or log-normal distribution characterized by a population mean and an interindividual variance. Preliminary analysis revealed that the data were best fitted by a one-compartment model (model producing the smallest log likelihood, the Akaike criterion, and the best fit of the data points), that the probability distribution of the random effect parameters was better described by log-normal rather than normal distribution, and that the residuals distribution showed that the error variance was better described by a heteroscedastic (proportional to the squared value of the predictions) model.

From the fitted parameters, (CL/F=clearance divided for the fraction of the dose absorbed (assumed=1), V=volume of the central compartment and Ka=absorption rate constant), other pharmacokinetics parameters were calculated: AUC(0-∞) (the area under the best-fitted curve of the plasma concentration-time data calculated by Dose.F/CL), C_{ss} (the average plasma concentration at steady-state calculated by AUC(0-∞)/τ where τ is the dosing interval), t_{1/2} (the elimination half-life).

The maximum plasma concentration (C_{max}) and the correspondent time (T_{max}) were determined by observation from each patient's data.

Statistical analysis and presentation. Data were stored, analyzed and reported with the packages STATISTICAL™ (StatSoft, Inc. Tulsa, OK), and Fig.P™ (Biosoft, Cambridge, UK), both run on a PowerExec EL (AST, Irvine, California) personal computer. Results were expressed as mean ± SD unless otherwise stated. Normal distribution of data was tested with the Shapiro-Wilk's W test. One-way analysis of variance was employed to detect significant differences between the 3 groups with respect to BU clearance and mean plasma concentration at the steady state, whereas the LSD test was employed to evaluate the differences between two groups. The Chi-square test was used to compare different frequencies. P values lower than 0.05 were considered to be statistically significant (27).

Results

The calculated pharmacokinetic parameters for the three

Table II. Pharmacokinetic parameters of busulfan.

PtN.	Dose (mg/kg)	C _{max} (ng/ml)	T _{max} (hours)	AUC (ng·h/ml)	C _{ss} (ng/ml)	CL/F (l/h·m ²)	T _{1/2} (hours)
Control group							
1	16.0	1064.90	0.5	4229.76	704.96	9.220	1.94
2	16.0	756.70	1	4073.15	678.66	9.703	2.02
3	16.0	1409.90	1	5240.25	833.71	7.575	2.10
4	16.0	1332.69	1	5240.81	1016.76	7.732	2.22
5	16.0	1180.38	2	4429.48	771.96	9.030	1.92
6	16.0	977.31	1	4125.63	687.60	8.864	2.09
7	16.0	1231.15	1	4233.88	705.65	9.185	2.04
8	16.0	986.15	2	4206.45	701.07	9.773	2.29
9	16.0	1045.00	2	4257.49	709.58	9.781	2.04
10	16.0	782.99	2	3384.55	564.09	10.949	1.74
11	16.0	1127.5	4	6454.94	1075.82	5.809	3.15
12	16.0	990.00	4	4579.59	763.26	8.324	2.10
13	16.0	613.37	2	3088.85	516.47	12.706	1.51
14	16.0	742.50	2	3410.87	568.48	11.063	1.76
15	16.0	621.92	4	4085.63	681.94	9.083	2.09
16	16.0	1401.05	0.5	4329.38	721.56	7.237	1.73
17	16.0	822.80	1	3621.09	603.51	9.365	2.07
18	16.0	643.21	2	3067.67	511.28	11.176	1.57
19	16.0	800.80	4	4957.37	826.23	5.896	2.59
20	16.0	309.69	4	2775.62	426.60	10.991	1.35
21	16.0	732.91	0.5	2963.55	493.92	9.945	1.45
22	16.0	824.45	1	3382.95	563.82	8.346	1.73
23	16.0	944.31	1	3376.21	562.70	8.885	1.65
24	16.0	649.43	0.5	2398.20	399.70	9.679	1.04
25	16.0	499.98	2	2111.71	351.95	11.012	1.09
26	16.0	268.80	2	2038.11	339.68	12.296	1.06
Mean	16.0	875.36	1.8	3839.81	639.97	9.373	1.86
SD	0.0	303.17	1.2	1011.30	168.55	1.702	0.47
Intracozazole							
1	16.0	817.36	4	4865.47	810.91	8.437	2.35
2	16.0	1379.9	1	6760.41	1126.73	6.150	3.28
3	16.0	2125.75	1	7829.63	1304.94	5.100	3.35
4	16.0	957.00	1	5074.34	845.72	5.912	2.17
5	16.0	1985.25	1	5187.53	864.59	7.447	1.53
6	16.0	951.92	1	3665.55	610.92	11.253	1.7
7	16.0	770.00	2	4267.49	711.25	9.226	2.18
8	16.0	1548.25	0.5	5739.21	956.53	5.888	2.27
9	16.0	975.86	2	4212.12	702.02	7.815	2.19
10	14.1	854.02	1	3826.87	637.81	9.648	1.72
11	15.2	709.50	1	5067.14	844.52	5.791	2.52
12	16.4	776.99	1	3259.13	543.18	7.451	1.68
13	18.2	1325.10	1	2518.65	419.77	9.453	1.23
Mean	16.0	1167.69	1.3	4790.27	798.38	7.653	2.17
SD	1.0	472.18	0.9	1433.12	238.85	1.871	0.63
Fluconazole							
1	16.0	1307.30	1	4911.83	857.24	7.940	2.05
2	16.0	1019.99	4	6382.43	1063.74	7.126	3.25
3	16.0	1347.50	2	4909.18	818.85	8.439	2.15
4	16.0	910.80	2	4125.41	687.57	9.532	2.14
5	16.0	305.99	1	3158.56	526.43	11.208	1.6
6	16.0	1127.50	1	4038.50	668.08	9.355	1.55
7	16.0	1713.25	0.5	4765.32	795.89	8.376	1.31
8	16.0	577.50	2	3259.03	543.17	11.455	1.65
9	16.0	398.20	0.5	3162.05	527.01	10.093	1.61
10	16.0	426.80	0.5	2645.85	440.98	10.994	1.34
11	16.0	515.60	4	1917.84	319.64	14.551	0.85
12	16.0	206.80	1	2367.70	394.62	10.030	1.18
13	16.0	267.66	2	1935.21	322.53	12.238	0.99
Mean	16.0	751.14	1.4	3658.38	609.73	10.103	1.67
SD	0.0	514.20	0.9	1338.82	223.04	2.007	0.63

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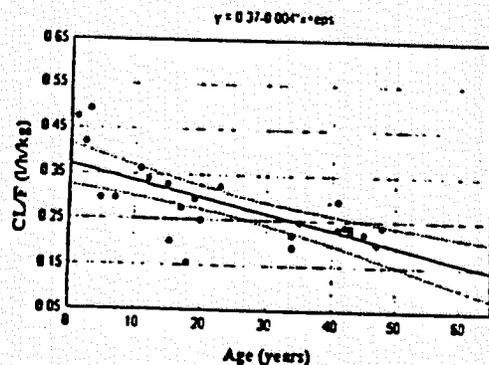


Figure 1. Correlation between CL/F corrected for weight and age.

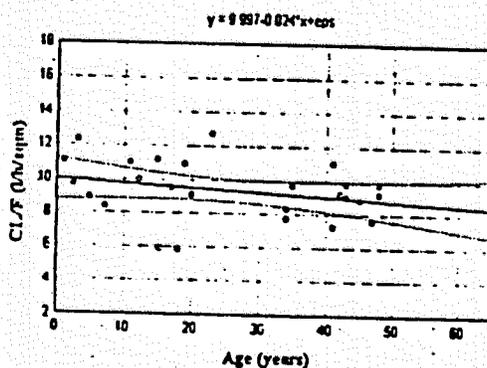


Figure 2. Correlation between CL/F corrected for BSA and age.

groups of patients are shown in Table II. In the control group (N=26), BU C_{max} was 875.38 ± 303.17 ng/ml, T_{max} was 1.8 ± 1.2 hours, AUC was 3938.81 ± 1011.30 ng.h/ml, C_{ss} was 639.97 ± 168.55 ng/ml, t_{1/2} was 1.86 ± 0.47 hours and CL/F was 9.373 ± 1.702 l.h/m².

Age-related pharmacokinetic differences were observed. In order to minimize the interindividual variability due to age-dependent clearance (the younger the patient, the higher the clearance rate (9-12), we normalized clearance for body surface area rather than body weight (28). The relationship between clearance normalized for body weight and age, and clearance normalized for body surface area and age are shown in Figures 1 and 2 respectively.

The thirteen patients of the itraconazole group gave a mean \pm SD BU C_{max} value of 1167.69 ± 472.18 ng/ml, T_{max} was 1.3 ± 0.9 hours, AUC was 4790.27 ± 1433.12 ng.h/ml, C_{ss} was 798.38 ± 238.85 ng/ml, t_{1/2} was 2.17 ± 0.63 hours and CL/F

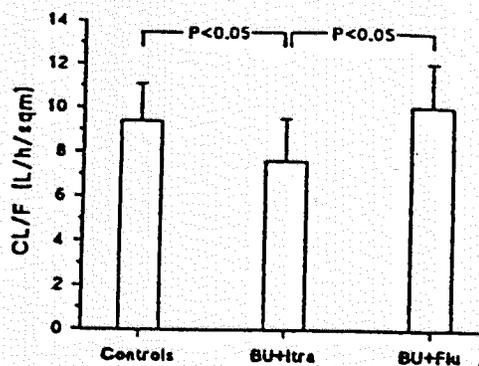


Figure 3. Influence of itraconazole and fluconazole on BU clearance. Data are expressed as mean and standard deviation.

was 7.653 ± 1.871 l.h/m². In patients receiving fluconazole (N=13) BU C_{max} was 751.14 ± 514.20 ng/ml, T_{max} was 1.4 ± 0.9 hours, AUC was 3658.38 ± 1338.22 ng.h/ml, C_{ss} was 609.73 ± 223.0 ng/ml, t_{1/2} was 1.67 ± 0.63 hours and CL/F was 10.103 ± 2.007 l.h/m².

The measured BU concentrations and AUC values increased in all patients given itraconazole but not in the fluconazole or control patients. The mean BU C_{ss} was higher in the itraconazole group (798.38 ± 238.85 ng/ml) as compared to the fluconazole group (609.73 ± 223.04 ng/ml) and the control group (639.97 ± 168.55 ng/ml) ($p < 0.01$).

The effect of itraconazole on BU pharmacokinetics was best reflected in BU clearance since BU dose was modified in some patients. In fact, in comparison to controls and patients receiving fluconazole, BU clearance was decreased by an average of 20% in patients receiving itraconazole (see Figure 3 showing the different clearance of BU in the three group of patients). MANOVA revealed a statistically significant difference between CL/F values for the itraconazole group in comparison with the other two groups (fluconazole and control patients) ($p < 0.01$).

Only 10 out of 26 controls (38%) and 3 out of 13 patients given BU and fluconazole (23%) experienced grade II-III regimen-related toxicity. In contrast, 9 out of 13 patients (69%) given BU had comparable toxicity ($p < 0.05$ in comparison to the fluconazole group and $P = 0.07$ in comparison to the control group).

Discussion

Imidazoles are thought to exert their antifungal effects by inhibiting lanosterol C-14 demethylase, a cytochrome P-450-metabolizing enzyme, responsible for converting lanosterol into ergosterol, a substance needed for the fungal cell membrane (29).

These drugs also bind and inhibit the hepatic cytochrome P-450-enzymes responsible for drug metabolism. Fluconazole affecting human enzymes to a lesser extent than itraconazole (or ketoconazole) (30-32). In this study, itraconazole, but not fluconazole, markedly affected the pharmacokinetics of BU. In fact, the concomitant use of itraconazole at the usual therapeutic dose resulted in a 20% decrease in BU oral clearance with a correspondent increase of BU plasma levels. The nature of this interaction, however, is unclear.

The chemical fate of BU has not yet been fully characterized. BU clearance is mainly extrarenal since less than 10% of the dose is excreted unchanged in urine within 24 hours (16,33). The drug is extensively biotransformed in the liver through conjugation to reduced glutathione (GSH) by glutathion-S-transferases in rodents and humans (14,34-40). The first and major metabolite is the sulfonium ion of glutathione, which is further transformed in 3 hydro-soluble metabolites (sulfolane, 3-hydroxy-sulfolane, tetrahydro-thiophene-1-oxide). Most studies in animals (mouse) have failed to show any involvement of oxidative metabolism enzymes (Cytochrome P450) in BU liver biotransformation (36). Vassal *et al* (1994)(37) reported that drug interactions with BU metabolism may occur by at least two means: depletion of liver GSH content and induction of glutathion-S transferases. Nonetheless, itraconazole and its analogues are inhibitors of both cytochrome P450 and lipooxygenase and since itraconazole modulates BU pharmacokinetics, oxidative metabolism is probably a determinant of BU metabolism. This hypothesis should be further investigated in human metabolic studies.

There is clinical evidence that variations in BU disposition may be responsible for differences in the toxic and therapeutic effect seen in patient population (41). Patients with slow rates of drug clearance would have a relatively high level of systemic exposure: with a corresponding high probability of unacceptable toxicity (42). In our study, the itraconazole patient group evidenced higher BU plasma levels than the control and fluconazole group, and this resulted in a higher incidence of regimen-related toxicity.

The clinical implication of these results is that clinicians should be aware that itraconazole may increase the BU plasma concentrations, possibly requiring dosage adjustment in the myeloablative therapy when the two drugs are administered concomitantly.

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