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Pharmacokinetics of high-dose busulfan in children

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Summary. The pharmacokinetics of high-dose busulfan given orally at 1 mg/kg every 6 h over 4 days (total dose, 16 mg/kg) in combined chemotherapy followed by autologous bone marrow transplantation was studied in 12 children with a mean age of 7 years (range, 4–14 years). Busulfan levels in biological fluids were measured by a gas chromatographic assay with mass fragmentographic detection, using a deuterated analogue as the internal standard. In a high-dose regimen, busulfan followed one-compartment model kinetics with zero-order absorption. A mean maximal concentration of 803 ± 228 ng/ml was achieved at 92–255 min after dosing. The mean elimination half-life was 2.33 h, and the mean total clearance was 119 ± 54 ml/min per m^2, with an apparent distribution volume of 27.10 ± 11.50 l/m^2. A mean trough level of 370 ng/ml was found throughout the 4 days of the chemotherapy course. There were no significant variations in pharmacokinetic parameters measured after the first and last doses. Busulfan was monitored in the CSF of nine children at 3.25–7 h after the last dose and was detected in all patients, with a mean CSF-to-plasma concentration ratio of 0.95 (range, 0.5–1.4).

Introduction

Busulfan, or 1,4-butanediol dimethanesulfonate, has been used for 10 years at high doses (16 mg/kg) in combined chemotherapy as an alternative to total body irradiation with cyclophosphamide as a preparative regimen before bone marrow transplantation [14]. Chemotherapy including high-dose busulfan has demonstrated an antitumoral effect in acute leukemia [18] and solid malignant tumors [8] and has been described as being an efficient myeloablative regimen for allogeneic or syngeneic bone marrow grafting [3, 11, 13, 17]. High-dose busulfan is frequently used in children because it exhibits fewer important, late side effects than total body irradiation.

Busulfan pharmacology has been more extensively studied since Ehrsson and Hassan [5] developed a gas chromatographic assay in plasma. Pharmacokinetic parameters for conventional low doses (2–6 mg) were established in adults [6], where busulfan kinetics could be described as fitting a one-compartment model with zero-order absorption. However, to our knowledge, there are no data for high-dose busulfan kinetics in children.

In this paper we describe and analyse the plasma busulfan profiles in children treated with high-dose busulfan at a dose of 1 mg/kg given orally every 6 h over 4 days, for a total dose of 16 mg/kg. Drug assay was carried out by gas chromatography and mass spectrometry (GC-MS) with selected ion monitoring (SIM) using a deuterated analogue, busulfan-d4, as the internal standard [20]. Because busulfan is highly lipophilic and since it triggers convulsions in animals [2] and patients [15] treated at high doses, we checked for busulfan distribution in the CSF.

Materials and methods

Patients. A total of 12 children with malignant disease were included in this study (Table 1): 6 had neuroblastoma, 2 had non-Hodgkin's lymphoma, 2 had acute lymphoblastic leukemia, 1 had malignant histiocytosis and 1 had Ewing's sarcoma. The median age at the time of bone marrow transplantation was 7 years (range, 4–14 years). None of the patients had ever had CNS involvement. Prior to chemotherapy, renal and hepatic functions were normal.

Treatment. Busulfan (Misulban; Laboratoire Techni-Pharma, Monaco Principality) was given orally at 1 mg/kg every 6 h as 2 mg tablets easy for a young child to chew or as a reconstituted capsule including the entire dose. No vomiting occurred during busulfan therapy, which was received on an empty stomach. Each patient received combined chemotherapy including 16 mg/kg busulfan (BU) given over 4 days, with high-dose melphalan (HdM, 140 mg/m^2) given as a 1-h i.v. infusion and/or cyclophosphamide (CPM) given at 200 mg/kg over 4 days as a 50 mg/kg daily i.v. infusion lasting 1 h (Fig. 1). Forced diuresis was maintained throughout the course by an i.v. infusion of 3 l/m^2 glucose in water (5%) with electrolytes. Autologous bone marrow transplants were infused 24 h after the last drug dose; ten transplants had been treated in vitro with Asta-Z (Asta Werke, FRG). Thus, eight children received HdM-BU-CPM, three received BU-CPM and one, HdM-BU.

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Table 1. Patient characteristics

Patient number	Age (years)	Sex	Tumor	Treatment
1	4	M	NB	HdM-BU-CPM
2	11	M	NHL	HdM-BU-CPM
3	5	M	NB	BU-CPM
4	5	M	NB	HdM-BU-CPM
5	14	F	ES	BU-CPM
6	14	M	NHL	HdM-BU-CPM
7	7	M	ALL	HdM-BU-CPM
8	6	F	NB	HdM-BU-CPM
9	4	M	NB	HdM-BU-CPM
10	5	M	NB	HdM-BU-CPM
11	4	M	MH	BU-CPM
12	7	M	ALL	HDM-BU

NB, neuroblastoma; NHL, non-Hodgkin's lymphoma; ES, Ewing's sarcoma; ALL, acute lymphoblastic leukemia; MH, malignant histiocytosis; HdM, high-dose melphalan; BU, busulfan; CPM; cyclophosphamide

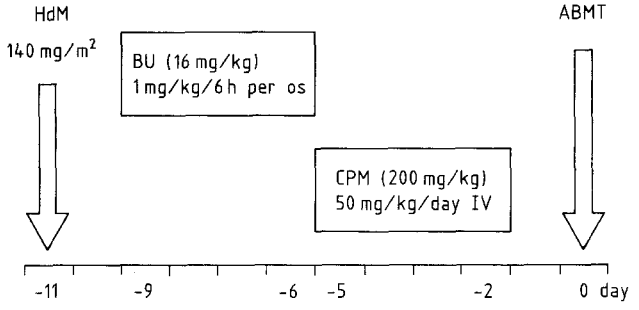

Fig. 1. Treatment schedule. *HdM*, high-dose melphalan; *BU*, busulfan; *CPM*, cyclophosphamide; *ABMT*, autologous bone marrow transplantation

Blood and CSF samples. Each child had a central catheter for supportive care during his aplasia, allowing frequent and painless blood sample collection. In 11 patients blood samples were drawn at 0, 20, 40, 60, 90, 120 and 150 min and 3, 4, 5, 6, 12, 24, 48, 72, 90, 92, 94, 96 and 102 h after the start of busulfan therapy and were immediately cooled at 4° C. Plasma samples were separated by centrifugation at 4° C within 1.5 h, then frozen and stored at −20° C. In ten patients CSF was drawn through lumbar puncture within 3.25–7 h after the last oral dose. CSF (1 ml) was immediately frozen and stored at −20° C.

Drug assay. Busulfan levels in plasma and CSF were measured by a gas chromatography assay on a capillary column with mass spectrometric detection [20]. Briefly, from 1 ml plasma previously spiked with 100 µl busulfan-d4 (internal standard) solution in acetone (2.5 µg/ml), busulfan was extracted into ethyl acetate (6 ml). The organic phase was collected and evaporated to dryness under nitrogen. The residue was reconstituted in 2 ml ethyl acetate and 1 ml 4 *M* aqueous solution of sodium iodide was added. The conversion of busulfan to the volatile 1,4-diiodobutane was carried out under stirring at 70° C for 30 min. The organic phase was then washed with distilled water (1 ml) and evaporated to dryness. After reconstitution in 20 µl acetone, 0.2 µl was injected into the GC-MS system. As described above, a deuterated analogue, busulfan [2,2,3,3-^{2}H], was used as the internal standard, which is extracted and converted to 1,4-diiodobutane [2,2,3,3,-^{2}H] in the same way and to the same extent as busulfan itself. Detection was done by MS-SIM focused on ion m/z = 182.9671 for 1,4-diiodobutane and ion m/z = 186.9222 for 1,4-diidobutane [2,2,3,3-^{2}H]. Retention times were 3.55 and 3.53 min, respectively. The detection limit of busulfan in plasma was 0.5 ng/ml.

Pharmacokinetic calculations. The kinetic analysis was carried out according to a one-compartment, openbody model with zero-order absorption. The kinetics were assimilated to an i.v. infusion that started at the absorption lag time, t_{lag}, with a constant infusion rate corresponding to the absorption rate constant, k_o, and finished at the time for the end of absorption, t_{max}.
The kinetic constants were evaluated according to:

$$C_t = \frac{k_o F}{K_e V} \quad 1 - e^{-ke(t - t_{lag})} \tag{1}$$

during the absorption time, and

$$C_t = \frac{K_o F}{K_e V} \quad 1 - e^{-ke(t_{max} - t_{lag})} \quad e^{-ke(t - t_{max})} \tag{2}$$

after the end of absorption, where C_t is the concentration in plasma at time t, k_o is the apparent zero-order absorption rate constant, K_e represents the overall elimination rate constant, V_d is the apparent distribution volume, F is the bioavailability, t_{lag} represents the absorption lag time, t_{max} is the time for the end of absorption and C_{max} is the maximal concentration. The absorption rate constant was calculated according to

$$k_o = \frac{\text{dose}}{t_{max} - t_{lag}}. \tag{3}$$

Pharmacokinetic parameter such as the AUC (log/linear trapezoidal rule), total clearance corrected for bioavailability (Cl/F), elimination rate constant K_e and elimination half-life $t_{1/2}$ were determined by computer nonlinear regression analysis of plasma concentration-time data using the PKCalc program [19], according to Eqs. 1 and 2.

This program enabled the calculation of the mean residence time, MRT, according to the equation

$$MRT = \frac{AUMC}{AUC} \tag{4}$$

where

$$AUMC = \int_0^\infty t\, C_t\, dt, \tag{5}$$

$$AUC = \int_0^\infty C_t\, dt, \tag{6}$$

and the apparent distribution volume according to

$$V_d/F = MRT - \frac{t_{max} - t_{lag}}{2} \quad \frac{Cl}{F}. \tag{7}$$

Results

Pharmacokinetic parameters determined after the first oral dose of 1 mg/kg busulfan are summarized in Table 2, and Fig. 2a shows the mean plasma concentration-time curve along with standard deviations. The absorption phase showed interindividual variations, with a t_{lag} in the range of 0.1–1.0 h, a t_{max} between 1.53 and 4.25 h and a mean

Table 2. Pharmacokinetic parameters in plasma after the first and last doses of 1 mg/kg busulfan in 11 children

Patient number	Dose (mg)	t_{lag} (h)	t_{max} (h)	C_{max} (ng/ml)	$t_{1/2}$ (h)	MRT (h)	AUC (ng·h/ml)	Cl/F (ml/min per m^2)	V_d/F (l/m^2)	$t_{1/2}$[a] (h)	C_{max} (ng/ml)[a]
1	12	0.66	3.00	738	1.60	5.02	3,222	107	24.80	2.71	901
2	28	0.90	4.25	378	1.61	5.03	1,652	282	56.85	1.64	554
3	16	0.25	1.66	817	1.91	3.76	3,103	126	23.15	2.23	710
4	20	0.17	2.03	1,176	2.55	4.70	5,505	80	18.25	2.94	1,115
5	45	1.00	4.00	821	2.17	5.57	3,875	129	31.50	ND	ND
6	32	0.33	2.50	1,108	2.80	5.53	6,120	85	22.70	3.56	828
7	20	0.33	2.50	935	2.26	4.35	4,613	90	17.70	2.26	1,238
8	15	0.10	2.00	830	2.84	4.80	4,437	86	20.00	2.65	585
9	12	0.30	1.53	734	2.30	4.19	3,455	102	21.40	2.39	640
10	20	0.12	2.05	864	2.18	4.27	3,880	100	20.00	2.63	935
11	14	0.42	3.16	440	3.37	7.14	3,238	120	41.50	2.92	631
Mean	21.3	0.41	2.60	803	2.33	4.94	3,918	119	27.10	2.59	819
SD	9.6	0.29	0.88	228	0.51	0.87	1,170	54	11.50	0.49	229

[a] Parameters measured after the last dose of busulfan

t_{lag}, absorption lag time; C_{max}, maximal plasma concentration achieved at time t_{max}; $t_{1/2}$, elimination half-life; MRT, mean residence time of the drug; AUC, area under the curve; Cl/F, total clearance corrected for bioavailability; V_d/F, apparent distribution volume corrected for bioavailability; F, bioavailability; ND, not done

C_{max} of 803 ± 228 ng/ml. For each patient the elimination phase was monophasic, with a mean half-life of 2.3 ± 0.5 h (range 1.60–3.37 h). Systemic clearance (Cl/F) was 119 ± 54 ml/min per m^2 (range, 80–282 ml/min per m^2) and V_d was 27.1 ± 11.5 l/m^2, where F represents the bioavailability. The AUCs and MRTs are given in Table 2; the means $\pm$SD was $3,918 \pm 1,170$ ng.h/ml and 4.9 ± 0.9 h, respectively.

Plasma levels of busulfan were detectable throughout the 4 days of busulfan therapy in all patients. Trough levels were in the range of 330 ± 180–447 ± 185 ng/ml (Fig. 2b). Repeated doses over 4 days did not modify busulfan kinetics or trigger important accumulation, since a comparison between pharmacokinetic parameters following the first and last doses revealed no significant difference. The elimination phase was also monoexponential, with respective mean half-lives of 2.33 h (first dose) and 2.59 h (last dose) and a mean C_{max} of 804 ± 229 (first dose) and 809 ± 229 ng/ml (last dose). On the other hand, there were intraindividual variations between the first and last doses as depicted in Table 2.

CSF busulfan levels were measured in nine children, none of whom had ever had CNS disease. Whatever the CSF collection time, busulfan was detected in all patients studied at levels in a range from 215 to 881 ng/ml, with a mean CSF-to-plasma ratio of 0.95 ± 0.25 (Table 3). No child developed any neurological toxicity during this study.

Discussion

In our patients, we monitored unchanged busulfan in plasma and CSF during a high-dose regimen that consisted of a total of 16 doses of 1 mg/kg given every 6 h over 4 days. After an oral dose of 1 mg/kg, i.e. 10- to 20-fold higher than conventional doses, busulfan follows first-order kinetics, with an elimination half-life of 2.33 h. Our results are very close to those of Ehrsson et al. [6], who studied busulfan kinetics in adults after low doses (total dose 2–6 mg). Contrary to other anticancer agents such as methotrexate or 5-fluorouracil [16], increased doses of busulfan do not modify the pharmacokinetic behavior of the drug.

After low and high doses, busulfan absorption proceeds as a zero-order kinetic process: a constant fraction of the dose reaches the central compartment per time unit. For galenic forms, the absorption rate constant is a composite of at least two mechanisms: (1) the dissolution of the drug in the gastrointestinal tract and (2) the absorption itself through the intestinal mucosa. Classic methods do not enable the deconvolution of these two processes. Nevertheless, Ehrsson et al. [6] have found that the mean values for the absorption rate constant and the time for the end of absorption are independent of the dose, in a range from 2 to 6 mg; these authors suggest that zero-order absorption may be a consequence of zero-order drug dissolution in the gastrointestinal tract.

In our study, eight children also received a 1-h i.v. infusion of melphalan (140 mg/m^2) 48 h prior to the first busulfan dose. In a previous paper [7], we established that in children, the plasma pharmacokinetics of melphalan after such a dose follow a biphasic pattern, with an elimination half-life of 48 ± 16 min (always < 80 min). We cannot rule out any drug interaction with either melphalan or cyclophosphamide, but our results are in good agreement with those of Ehrsson et al. [6], as cited above.

Throughout the 4 days of therapy, a mean trough concentration of 370 ng/ml was achieved. There were no significant modifications of the kinetics after 4 days of therapy, since the mean values of C_{max} and half-lives after the first and the last doses were very similar. This stability of the pharmacokinetic parameters after repeated doses suggests a lack of dose dependency and/or enzyme induction or inhibition that could have altered the biotransformation rate. On the other hand, these results prove that during a high-dose regimen, busulfan is present in the central compartment for 102 h, with a concentration peak every 6 h. The large V_d observed in children leads us to believe that the active principle can reach each organ as well as the tumor or its metastases for a long residence time.

This is confirmed by the fact that after 4 days of therapy, busulfan was detected in CSF in all patients studied,

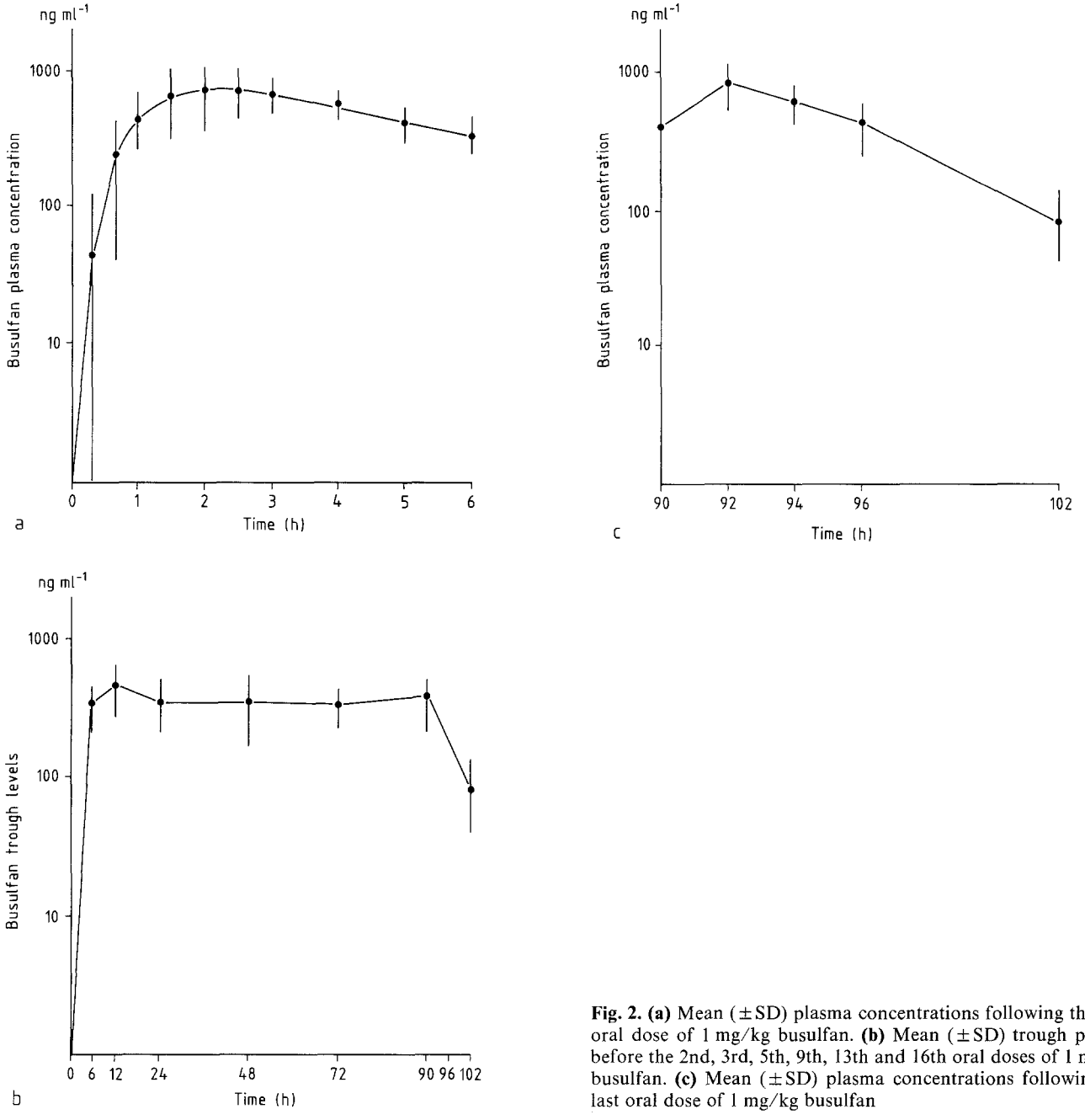

Fig. 2. (a) Mean (±SD) plasma concentrations following the first oral dose of 1 mg/kg busulfan. **(b)** Mean (±SD) trough plasma before the 2nd, 3rd, 5th, 9th, 13th and 16th oral doses of 1 mg/kg busulfan. **(c)** Mean (±SD) plasma concentrations following the last oral dose of 1 mg/kg busulfan

Table 3. CSF busulfan levels after the last oral dose of 1 mg/kg in nine children

Time[a] (h)	Patient number	CSF (ng/ml)	Plasma (ng/ml)	*r*
93.25	7	874	1500	0.60
93.50	6	466	800	0.60
94.00	10	626	459	1.40
94.00	2	429	379	1.10
94.00	1	739	660	1.10
94.50	4	881	1500	0.60
95.00	11	303	301	1.00
96.00	12	320	338	0.95
97.00	9	215	240	0.90

[a] Time after the start of busulfan chemotherapy
r, ratio of CSF to plasma

whatever the time of the lumbar puncture. Busulfan levels in CSF were high, with a mean CSF-to-plasma ratio of 0.95±0.25. No child had ever had CNS malignant disease that would have facilitated busulfan transfer through the blood-brain barrier. High-dose regimens allow some anticancer drugs to cross the blood-brain barrier, but the extent of this transfer is rather less important. For example, after a 24-h i.v. infusion of methotrexate at a dose ranging from 0.5 to 33.6 g/m^2, drug CSF levels are 1%–5% of plasma levels [4]. After high-dose melphalan, the drug is detected in CSF only in 37% of children [7]. These results are in agreement with the fact that busulfan is a highly lipophilic agent, able to cross physiological barriers and plasma membranes. This observation raises additional questions: (1) what is the kinetic profile of busulfan in CSF? (2) Is it dose-dependent? (3) Are convulsions ob-

served during a high-dose regimen [15] attributable to accumulation due to a low CSF clearance or individual sensitivity? Regardless of the answers, busulfan chemotherapy may be reconsidered for the treatment of some tumors in children in whom the CNS is a frequent disease sanctuary.

We could also detect unchanged busulfan in a pleural effusion after the last oral dose in a 27-year-old woman treated for non-Hodgkin's lymphoma. The pleural: plasma drug ratio was 0.85 (data not shown). Finally, although busulfan can be monitored in biological fluids by different methods (GC [9], GC-MS [5] or HPLC [10]), our GC-MS assay [20] using a deuterated analogue as the internal standard can be further developed for its application in metabolic studies in man [12] using stable isotopes [1].

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