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Pharmacokinetics of high-dose busulphan in relation to age and chronopharmacology*

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Summary. Busulphan levels in plasma were measured in 27 patients during conditioning therapy (1 mg/kg × 4 for 4 days) before bone marrow transplantation. The mean minimal concentration found in children aged <5 years (237 ng ml^{-1}) was lower than that observed in adults or older children (607 and 573 ng ml^{-1}, respectively). The AUC for the last dose was significantly lower in young children (2,315 h ng ml^{-1}) than in adults or older children (6,134 and 5,937 h ng ml^{-1}, respectively). The elimination half-life for the last dose in young children was shorter (2.05 h) than that in either adults (2.59 h) or older children (2.79 h). When the AUC was normalized for body surface area, the difference between young children and the other groups was smaller but remained statistically significant. The total body clearance was significantly higher in young children (7.3 ml min^{-1} kg^{-1}) as compared with both older children and adults (3.02 and 2.7 ml min^{-1} kg^{-1}, respectively). The plasma levels of busulphan showed circadian rhythmicity, especially in young children. The concentration measured during the night in some patients was up to 3-fold that observed during daytime. We conclude that the busulphan dosage for children must be reconsidered and that further studies are urgently needed to develop an optimal therapy.

Introduction

Allogeneic and autologous bone marrow transplantations have become efficient modes of therapy for the treatment of haematological malignancies [1, 6]. High-dose busulphan was introduced [18] as a myeloablative therapy substituting for total body irradiation (TBI) prior to bone marrow transplantation (BMT). Busulphan dosage prior to BMT is based on body weight (generally 1 mg/kg × 4 for 4 days). The pharmacokinetics of high-dose busulphan in adult patients have recently been reported [11], and a low level of interpatient variation was shown. Similar results have been obtained in paediatric patients [8, 19]. However, Hobbs et al. [12] reported that a better engraftment was achieved when the dosage was based on surface area. The well-known dosage problem in cancer chemotherapy has been described in detail for many anticancer agents [3].

Despite several years of experience with high-dose busulphan, very little is known about the disposition, efficacy and chronopharmacology of the drug. Several antineoplastic agents have shown age-related changes in pharmacokinetics [2]. This relationship might alter the efficacy and the toxicity profile of the drug, since it is well known that the interpatient pharmacokinetic variability is high in paediatric patients due to age-related maturation of physiological processes. The importance of chronopharmacology in drug therapy has recently been reported [15, 17], especially in attempts to minimize at least part of the side effects produced by anticancer agents [13].

In the present report we describe the relationship between the pharmacokinetics of busulphan and age in three patient groups: children aged ≤5 years, children aged 5–16 years and adults aged >16–50 years. We also report the circadian rhythmicity of busulphan. All patients were conditioned with busulphan (1 mg/kg p.o. × 4 for 4 days) followed by cyclophosphamide (60 mg/kg for 2 days or 50 mg/kg for 4 days) prior to BMT.

Patients and methods

Patients. Clinical data on 9 children are summarized in Table 1 and those on 18 adult patients are listed in Table 2. All patients showed normal liver and renal functions except patient 21, who exhibited a high transaminase level. The preparatory chemotherapy regimen before BMT con-

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Abbreviations: AML, acute myeloblastic leukemia; ALL, acute lymphatic leukemia; AUL, acute undifferentiated leukemia; ABMT, autologous bone marrow transplantation; BMT, allogeneic bone marrow transplantation

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Table 1. Clinical data for children

Patient number	Age (years)	Sex	Weight (kg)	Dose (mg)	Diagnosis	Transplantation (type)
1	1.3	M	12.5	12.5	ALL	ABMT
2	2	F	10.3	10	AML	ABMT
3	2	M	14.5	14	AML	ABMT
4	5	F	21.1	22	AML	ABMT
5	7	M	36.4	37.5	AML	ABMT
6	9	F	29.3	29	MLD	BMT
7	9	F	28.6	29	ALL	BMT
8	13	M	42.2	41	AML	ABMT
9	13	F	48.3	50	AML	ABMT

MLD, Metachromatic leukodystrophy

Table 2. Clinical data for adult patients

Patient number	Age (years)	Sex	Weight (kg)	Dose (mg)	Diagnosis	Transplantation (type)
10	19	F	55	50	AUL	ABMT
11	30	F	52	52	AML	ABMT
12	32	F	63	62	AML	ABMT
13	33	F	82	79	AML	ABMT
14	35	M	71	70	AML	ABMT
15	35	F	50	50	AML	BMT
16	35	M	91	91	AML	ABMT
17	35	F	86	79	AML	ABMT
18	36	F	58	56	AML	ABMT
19	37	F	59	60	ALL	BMT
20	38	M	100	100	AML	ABMT
21	39	M	72	75	Lymphoma	BMT
22	44	F	63	62	MDS	ABMT
23	45	M	82	81	AML	ABMT
24	48	F	74	75	AML	ABMT
25	48	F	81	70	AML	ABMT
26	48	F	85	81	AML	ABMT
27	50	M	72	75	AML	ABMT

sisted of high-dose busulphan (1 mg/kg p.o. g-6 h) given for 4 days followed by cyclophosphamide (60 mg/kg i.v. for 2 days or 50 mg/kg i.v. for 4 days). Busulphan was given as tablets of 2 and/or 25 mg (Wellcome Foundation Ltd. UK); in three of four children ≤5 years old, it was given as a water suspension via a gastric catheter. Special care was taken in washing the catheter with sterile water (10–20 ml) after each administration, no busulphan adsorbed on the surface of the catheter (data not shown).

Sample collection. Blood from adult patients (4–7 ml) and from children (1–2 ml) was taken from a central venous catheter immediately before and at various times during the treatment. The blood was collected in glass tubes (Vacutainer) containing 150 IU heparin. The plasma was separated by centrifugation at 2,000 g and stored at –20° C until assay. The samples were taken more frequently (10–15 samples) during the 1st and the 16th dose, whereas only one sample was drawn immediately before the administration of doses 2–15.

Busulphan determination. Busulphan in plasma was converted to 1,4-diiodobutane by reaction with sodium iodide in the presence of *n*-heptane and was measured by gas chromatography with electron-capture detection [9].

Pharmacokinetic and statistical analysis. The area under the plasma concentration-time curve (AUC) for the last dose was calculated using the trapezoidal rule. The total body clearance for each individual was calculated from the last dose and AUC. The elimination half-lives were determined from the linear portions of the log plasma AUC curves. The patient population was divided into three groups for statistical analysis: young children aged ≤5 years (A), older children aged between 5 and 16 years (B) and adults aged >16 years (C). The data for the different groups was analyzed by one way analysis of variance (Tukey HSD). Moreover, the pooled data obtained at each sampling time (from the achievement of steady state until dose 16) for the different groups during the entire therapy was analyzed using one-way analysis of variance.

The chronopharmacokinetics of busulphan were evaluated by comparing the mean daytime concentrations (1100–1400 hours over 4 days) with the mean nighttime concentrations (2300–0200 hours) for each individual after achievement of the steady-state level. The concentrations were calculated as a percentage of the mean minimal concentration measure during the steady state so as to standardize the plasma levels for all patients. Significance was established by one-sample analysis based on the individual differences between day and night. The continuous decrease in steady-state levels was evaluated using linear regression analysis. The percentage of decrease was calculated from the regression line as the ratio between the plasma level at dose 16 and that at dose 3.

Results

The maximal plasma concentration (c_{max}), the time to c_{max} (t_{max}) and the elimination half-life ($t^1/_2$) for the first and the last dose during busulphan therapy are given in Table 3. Patient 21 was excluded from the statistical analysis of all data presented below due to extremely high levels of busulphan with a mean minimal concentration of 1,660 ng/ml being measured after actievement of the steady-state level. This might be attributable to a liver-function disturbance that the patient experienced before receiving busulphan therapy. Moreover, interactive outlayer rejection analysis showed that patient 21 could be classified as an outlayer. Group A (children aged ≤5 years) showed more rapid drug elimination after the last dose ($t^1/_2 = 2.05 \pm 0.14$ h) than group B or C, which exhibited elimination half-lives of 2.74 ± 0.26 and 2.59 ± 0.09 h, respectively. Analysis of variance showed a significant difference ($P = 0.042$) between the groups.

The minimal concentrations of busulphan were measured immediately before the next dose. The mean minimal value during steady state (doses 3–15) was calculated for each patient (dose and body weight corrected to 1 mg/kg; Table 3). Figure 1 shows the mean minimal concentration for the individual patients in relation to age. The young children (age <5 years) showed lower plasma concentrations than either the older children or the adults. Analysis of variance revealed a significant difference between the groups ($P = 0.0019$).

The mean plasma concentrations for the three patient groups (data were pooled for each group) were also calculated for each sampling time during the entire 4-day treatment period and are illustrated in Fig. 2. At steady state, group A showed lower plasma levels, displaying a mean minimal concentration of 237 ± 10 ng ml^{-1} ($n = 13$), as compared with groups B and C (567 ± 15 and 607 ± 10 ng ml^{-1}, $n = 13$, respectively). Analysis of variance revealed a significant difference at $P < 0.0001$.

The AUC for all patients after the last dose is presented in Table 3. Group A showed a lower AUC ($2{,}315 \pm 149$ h ng ml^{-1} than group B ($5{,}937 \pm 891$ h ng ml^{-1}) or group C ($6{,}135 \pm 299$ h ng ml^{-1}). Statistical analysis revealed a significant difference ($P = 0.0001$) between the groups;

Table 3. Busulphan pharmacokinetic parameters in small children (A), older children (B) and adults (C)

Group	Patient number	First dose			Last dose				Mean minimal concentration[a] (ng/ml)	*C*/f (ml min^{-1} kg^{-1})
		c_{max} (ng/ml)	t_{max} (h)	$t^1/_2$ (h)	c_{max} (ng/ml)	t_{max} (h)	$t^1/_2$ (h)	AUC (h ng m^{-1})		
A	1	654	1	1.9	835	0.5	1.97	2,403	384	6.93
	2	461	1.5	ND	834	1	1.7	1,900	106	8.59
	3	1,466	0.25	3	1.057	0.5	2.17	2,348	212	6.85
	4	1,026	0.75	2.1	1,266	0.5	2.36	2,610	232	6.65
B	5	1,785	2	2.7	2,720	0.5	3.59	9,312	796	1.84
	6	1.610	2	2.4	1,468	2	2.48	4,911	450	3.35
	7	1,793	2	ND	2,711	1	2.58	5,618	636	3
	8	1,118	0.75	2	1,291	1	2.09	4,127	365	3,92
	9	2,003	0.5	3.2	2,641	0.5	2.99	5,718	616	3.01
C	10	1,109	0.75	2.1	1,450	2	2.77	5,932	579	2.55
	11	1,229	0.75	ND	2,313	1	ND	ND	539	ND
	12	1,955	0.5	1.8	1.340	3	1.94	4,546	396	3.59
	13	1,707	2	2.5	1,604	3	2.35	6,893	959	2.33
	14	1,943	0.25	3.1	1,976	2	3	6,898	911	2.36
	15	931	2	2.4	1,624	2	2.17	6,370	348	2.6
	16	1,345	1	2.7	2,162	1	3.18	6,583	583	2.53
	17	3,208	0.5	4.8	3,800	0.5	2.53	5,626	482	2.71
	18	1,380	1	2.1	1,737	0.75	2.31	4,793	475	3.38
	19	1,508	0.75	3.1	1,818	2	2.57	6,419	558	2.65
	20	1,431	4	2.2	1,490	4	3.38	ND	831	ND
	21	1,636	1.5	3.5	3,117	2	4.33	12,304	1,664	1,41
	22	1,214	3	2.1	2,258	2	2.64	5,314	506	3.11
	23	958	5	ND	1,533	1	2.58	6,857	620	2.39
	24	2,518	1.5	2.6	2,823	0.75	2.53	6,917	580	2.43
	25	727	4	5.7	894	3	2.64	3,978	550	3.6
	26	1,947	1.5	2.2	2,240	2	2.17	8,621	825	1.84
	27	1,399	0.75	3.4	1,463	1	2.7	6,274	581	2.76

ND, Not determined due to the insufficient number of levels measured; c_{max}, maximal plasma concentration; t_{max}, time to c_{max}; $t^1/_2$, elimination half-life; *C*/f, total body clearance based on the assumption that f = 1

[a] For doses 3–15

moreover, the difference remained significant ($P = 0.001$) following correction for surface area and dose.

The bioavailability (f) of busulphan is not known. Assuming that f = 1, the clearance (*C*/f) values, corrected for body weight, could be calculated from the last AUC and dose (Table 3). One-way analysis of variance revealed a significant difference between the groups ($P < 0.0001$). The level of significance between groups remained significant after clearance values had been corrected for surface area, as shown in Fig. 3. The mean *C*/f value (corrected for body surface area) for group A was 178 ± 8 ml min^{-1} m^{-2}), whereas those for groups B and C were 90 ± 10 and 105 ± 5 ml min^{-1} m^{-2}, respectively.

Regression analysis of the plasma levels obtained for patients who showed a continuous decrease during steady state is illustrated in Table 4. About 35% of the adult

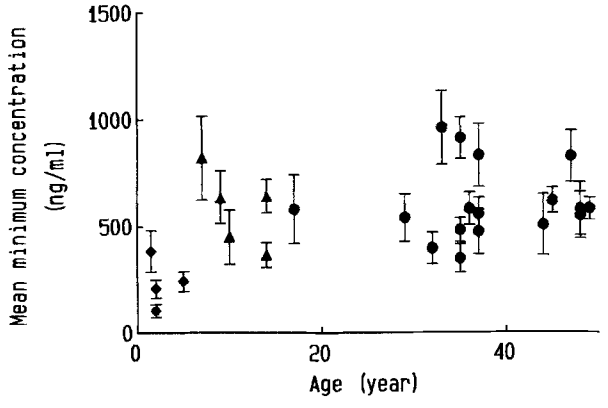

Fig. 1. Mean minimal plasma concentration of high-dose busulphan in all patients in relation to age. The concentrations are adjusted to a dose of 1 mg/kg. ◆, Young children; ▲, older children; ● adults

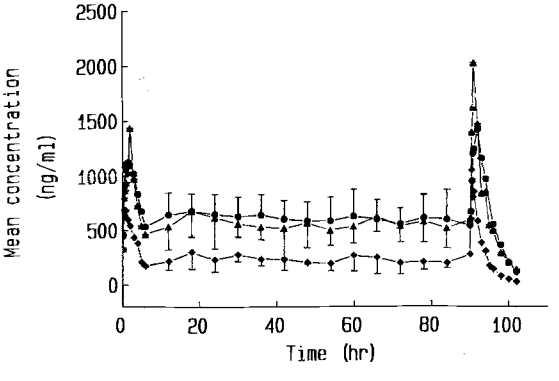

Fig. 2. Busulphan mean concentrations in plasma (pooled data from the three patient groups) during 4 days of treatment. The levels were adjusted to a dose of 1 mg/kg. ◆, Young children; ▲ older children, ● adults

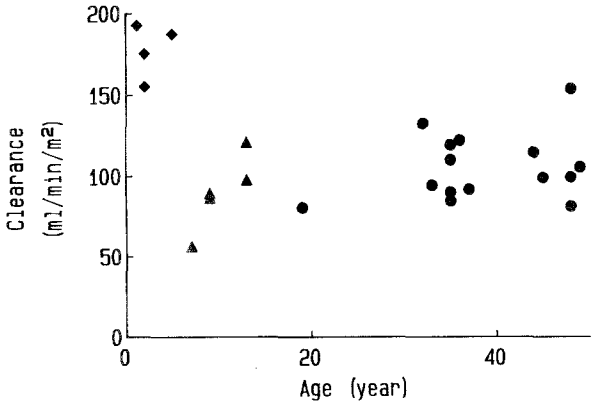

Fig. 3. Total body clearance (*C*/f), corrected for surface area) for all patients in relation to age. The values were calculated from the last dose and the AUC, assuming that f = 1. ◆, Young children; ▲, older children ●, adults

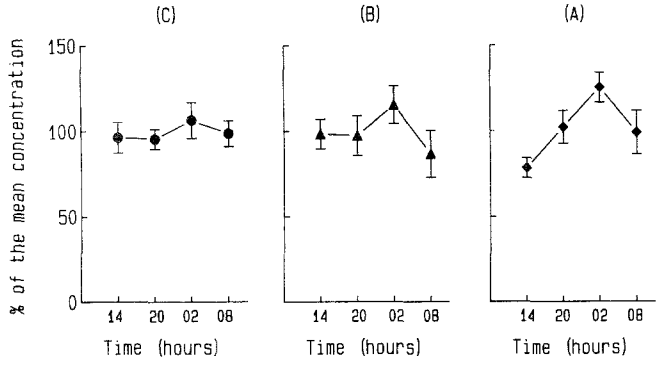

Fig. 4 A–C. Circadian rhythmicity during the 4 days of therapy. The mean daytime and nighttime concentrations during the treatment period were calculated as a percentage of the mean minimal concentration during the steady state. **A** Young children. **B** Older children. **C** Adults

patients showed a decrease of 30%–60% in their steady-state levels between dose 3 and dose 16. A decrease in steady-state concentration was also observed in young children (two cases) and in one of the older children. However, no increase in the steady-state level was observed during treatment in any of the patients.

A circadian rhythmicity was observed during the 4-day period of treatment with busulphan (Fig. 4). this pattern was significant ($P < 0.01$) in young children (group A), with the mean concentration during daytime being 78.4% ± 5.7% of the mean minimal value measured during steady state, whereas the mean nighttime concentration was 125.2% ± 8.9%. This rhythmicity was still pronounced and significant ($P < 0.01$) in older children (group B), who showed a mean daytime concentration of 86.6% ± 13.8% and a mean nighttime value of 115.2% ± 11%. In adult patients, no significant difference between the daytime and nighttime concentrations was observed.

Discussion

Dosage based on body weight is the standard procedure for high-dose therapy with busulphan [18]. In this respect busulphan differs from many other cytotoxic agents such as

Table 4. The decrease in the steady-state level with repeated administration of busulphan in young children (A), older children (B), and adults (C)

Group	Patient number	Intercept (ng/ml)	Slope	Significance level (*P*)	Decrease after 16 doses (%)
A	3	268	–1.17	0.012	42
	4	293	–1.04	0.08	34
B	9	759	–2.3	0.015	30
C	11	736	–3.80	0.004	50
	13	1,010	–2.55	<0.0001	24
	18	693	–4.27	<0.00001	49
	19	704	–2.32	0.003	32
	24	723	–2.57	0.07	34
	25	659	–2.55	0.09	25

bleomycin, anthracyclines and methotrexate [2], whose dosage is mainly based on body surface area. However, it has recently been reported that better engraftments in BMT were achieved when children with inborn errors of metabolism were conditioned with doses of busulphan based on body surface area [12].

In the present study, we found that children aged ≤5 years showed lower levels of busulphan during the entire treatment, shorter elimination half-lives after the last dose, and a lower AUC for the last dose than did adults or older children. The lower AUC and plasma values found for busulphan might be due to the differences in bioavailability, clearance or distribution volumes between children and adults. Age-related pharmacokinetics have previously been observed for other anticancer drugs. Methotrexate pharmacokinetics showed an age dependence that was attributed to greater drug distribution and clearance in young children [20]. Bleomycin was eliminated more rapidly by young children than by adults or older children [22].

The shorter elimination half-life for busulphan observed in young children in the present study might be explained by their higher total body clearance (assuming the same f value between the different patient groups). This is in good agreement with the clearance results recently reported by Grochow et al. [8]. We have previously shown that busulphan is extensively metabolized in the rat liver [10] and that at least a part of busulphan is metabolized via the glutathione pathway in man [11]. The faster elimination half-life observed in young children may be attributable to higher levels of glutathione or glutathione S-transferase such as those occurring in premature infants and neonates [4]. However, to our knowledge, no study of glutathione levels in young children has been published.

Another possible explanation for the observed decrease in elimination half-life would be a decrease in the actual distribution volume in young children. Busulphan is known to be lipophilic. Since young children usually have more body water than adults [16], a lower distribution volume might be expected in young children than in adults. It has been shown that the total body water and, especially, the extracellular water is proportional more to body surface area than to body weight in young children [5].

A continuous decrease in steady-state drug levels was found in 35% of the adult patients, whereas only one of the older children and two of the young children showed this

tendency, which might be a result of induction of glutathione or its transferases in these patients. The continuous decrease in steady-state levels in adults has previously been suggested to be due to autoinduction by busulphan of its own metabolism [11].

In the present investigation a circadian rhythmicity for busulphan was observed. There was up to a 3-fold variation between the daytime and the nighttime concentrations; however, it is too early to speculate about the possible clinical importance of this observation. For other cytotoxic agents such adriamycin and cisplatin, treatment complications could be reduced by 50% when the administration time was considered [13]. It has also been shown that etoposide is better tolerated in mice when the drug is given in the second half of the sleeping span [14]. However, it is important that the daily variation in busulphan concentrations be considered when dose adjustment is based on therapeutic drug monitoring [7].

This study demonstrates that the AUC for the last dose was lower for young children than for adults. This difference in AUC was smaller but remained measurable when AUC was correlated to body surface area. Moreover, a significantly higher total body clearance was observed in the young children than in either the adults or the older children. This difference remained significant after the values had been corrected for body surface area. At a given dosage, the plasma levels of busulphan corrected for body weight were found to be lower in young children than in adults during the 4 days of therapy with busulphan. It might therefore be more appropriate to correlate the dosage of busulphan to body surface area in young children. However, special caution must be taken since the toxicity of some anticancer drugs is higher in children with body surface areas of $<0.5\ m^2$ [21].

Further studies of the mechanisms contributing to these differences are necessary before therapy with busulphan can be individually optimized. Moreover, it is of great importance that the plasma levels of busulphan be correlated with the rates of relapse and long-term survival.

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