Toxicity and pharmacokinetics of i.v. busulfan in children before stem cell transplantation

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We have introduced a dimethylacetamide-based i.v. formulation of busulfan into pediatrics with a dose intensity [as measured by area under curve (AUC)] comparable to that achieved by oral busulfan while reducing variability. The target AUC was defined at 1600 ± 600 μM·min. The children received 15 doses of i.v. busulfan as 2-h infusions with a dose calculated to be 80% of the oral dose according to the malignancy-related protocol. The first infusion was applied as a double dose over 4 h with the second infusion following 12 h thereafter. Plasma samples were analyzed for busulfan by a validated LC-MS method and toxicity was assessed at least up to day 100 + after transplantation. Nineteen children (median age: 4 years, range: 0.9–17.3) were included. The AUC after the first dose ranged from 570 to 1410 μM·min [geometric mean 1010 μM·min, coefficient of variation (CV) = 22%, *n* = 17]. In nine out of 17 patients, the AUC after the first dose was out of the target range. Two patients had neurotoxic symptoms, which were attributable to busulfan in one individual. No case of severe hepatic veno-occlusive disease or other serious toxic events occurred. We conclude that i.v. busulfan displays a smaller interpatient variability in exposure compared to oral busulfan (CV of 24% after i.v. versus CV of 37% after oral busulfan). The equivalent dose to 1 mg/kg oral busulfan with regard to the AUC appears to be higher than 0.8 mg/kg. *Anti-Cancer Drugs* 16:337–344 © 2005 Lippincott Williams & Wilkins.

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Introduction

High-dose busulfan is an important element of many conditioning regimens given prior to stem cell transplantation in adults and children for hematological malignancies [1–5] and certain disorders [6–8]. The use of oral busulfan in high-dose chemotherapy in children is an 'off-label use' as it is not approved in this patient group. One problem is the high inter-individual variability in exposure after administration of oral busulfan in children: the area under the curve (AUC) range of oral busulfan is comparatively wide. About 10% of the patients achieve insufficient plasma concentrations, mainly due to slower absorption of busulfan and a rapid clearance [9]. On the other hand, a high exposure increases the risk of hepatotoxic effects with veno-occlusive disease (VOD) as the dose-limiting toxicity.

Dosing according to body surface area (BSA) in children has been shown to produce AUC values similar to those observed in adults [10,11]. However, children receiving BSA-dosed oral busulfan still show high inter-individual variability in the distribution, elimination and systemic availability of busulfan. Various pediatric studies have confirmed that the pharmacokinetics of high-dose busulfan strongly depend on age. Children have a 2–4 times higher total clearance and thus lower AUC than adults [10,12–18]. The usual dosage of 16 × 1 mg/kg body weight over 4 days (16 doses) is associated with a lower systemic availability of busulfan in children compared to adults [14,15,19].

Apart from the pharmacokinetic parameters, the formulation of the drug plays an important role. Many pediatric

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patients find it difficult to swallow busulfan tablets or capsules for oral use. The children complain of nausea after taking busulfan tablets. Consequently, at dosages of up to 30 tablets 4 times daily, a negative attitude towards oral medication for high-dose treatment is not surprising.

The introduction of busulfan for i.v. infusion provides patients with a drug formulation relieving them from the difficulty of swallowing. In addition, due to direct systemic application by infusion, the variability of systemic exposure, measured as AUC, should be reduced. The busulfan formulation for i.v. application (Busulfex; Orphan Medical, Minnetonka, MN) has been approved for high-dose therapy in combination with other drugs. In adults, some studies using this formulation were reported [20,21]. In children, up to now only very small studies have been conducted, showing that this formulation appears to be applicable in this patient group [22]. Nevertheless, studies in larger pediatric patient populations are lacking.

In the study presented here, i.v. busulfan containing *N*,*N*-dimethylacetamide (DMA) as a solvent was administered. Not only busulfan, but also DMA displays neurotoxic and hepatotoxic effects. Possibly, these toxic effects of DMA reported from animal models and older studies in humans [23–25] might be a problem, as children receive high cumulative doses of DMA in combination with busulfan.

Therefore, the objective was to monitor the toxicity and the pharmacokinetics of this i.v. busulfan formulation (Busulfex) in conditioning regimens prior to autologous or allogeneic stem cell transplantation.

Patients and methods

This study was approved by the local ethics committee. From March 2001 to September 2002 pediatric oncological patients and patients with congenital metabolic disease or immunodeficiencies younger than18 years were enrolled in the study. Written informed consent had to be given by all patients and parents, and a thorough examination had to be performed. Exclusion criteria were withdrawal of consent, contraindications to busulfan and exclusion criteria of the specific clinical trials the patients were enrolled on according to their malignancy or metabolic disease.

The trial was designed as a prospective open, multicenter trial at the University Hospital Münster, and additional transplant units could contribute first on a 'compassionate use' mode and be accredited as a trial center before, while or after treating the first patient. The projected number of patients was at least 15 within a period of 24 months.

In order to define the target AUC on the basis of studies using oral busulfan, a study in 27 children reported by Vassal *et al.* [10] was used. They reported a mean AUC of 1600 μM·min and a coefficient of variation (CV) of 37% (geometric mean = 1500 μM·min, geometric CV = 36%) after oral administration of 600 mg/m^2 busulfan in this group.

The primary endpoints of this study was, therefore, a target AUC of 1600 ± 600 μM·min with a CV smaller than or equal to 37%. In addition, the documentation of toxicity of i.v. busulfan was defined as another primary endpoint. Determination of the pharmacokinetic parameters volume of distribution (V), half-life ($t_{1/2}$), AUC, clearance (Cl), and the comparison of day 1 and 4 kinetics were investigated as secondary endpoints.

The first interim analysis had to be performed after accrual of five patients. In case of an AUC above 2200 or below 1000 μM·min in more than two patients, the study had to be stopped and the decision whether to continue or modify the study had to be taken by the drug safety committee.

Drug administration and dosage form

The dose for i.v. administration was calculated to be 80% of the usual oral dose. That implies that a single oral dose of 37.5 mg/m^2 or 1 mg/kg of busulfan had to be replaced by a dose of 30 mg/m^2 or 0.8 mg/kg busulfan i.v.

Busulfan was administered i.v. on 4 consecutive days. Patients received a total of 15 doses of i.v. busulfan (three doses on day 1 and four doses each on days 2–4).

In order to assess the elimination of busulfan over a longer time period, the first infusion was administered as a loading dose, giving a double dose over 4 h (e.g. 60 mg/m^2 or 1.6 mg/kg), followed by the second dose 12 h later. The second and all following doses (30 mg/m^2 or 0.8 mg/kg) were administered as 2-h infusions every 6 h. Busulfan was given through a multilumen central line via an infusion pump or syringe infusor.

Blood sampling and drug analysis

Blood samples were collected for analysis from the central venous line at 3, 4 (end of the infusion), 4.5, 5, 6, 8 and 12 h after beginning the first infusion at day 1 of treatment, and trough values prior to the fourth and eighth dose. In addition, blood samples were drawn 0 h (prior to infusion), 2, 3, 4 and 6 h after starting the 13th, 14th or 15th dose.

Samplings prior to the fourth dose and 2, 3, 4 and 6 h after starting infusion of the 13th, 14th or 15th dose were optional, and could be omitted, e.g. in small children. The heparinized blood was centrifuged within 45 min

after sampling and the resulting plasma samples were stored at –80°C until analysis.

Samples were analyzed for busulfan by a validated LC-MS method [26]. The assay requires only 200 µl of plasma, using [$^2H_8$]busulfan as the internal standard. After liquid–liquid extraction with diethylether, busulfan and [$^2H_8$]busulfan were detected as ammonium adducts in selected-ion monitoring mode. Linearity was shown over a concentration range of 5–2000 µg/l busulfan. Intra- and interassay imprecision (CV) and bias were both below 11%.

Statistical and pharmacokinetic analysis

The analysis included all patients treated with the test drug where clinical report forms have been submitted. Violations of the study plan were to be identified and recorded. The statistical analysis was performed using the software Sigmastat 2.03 (SPSS, Erkrath, Germany).

Trough levels (prior to the fourth, eighth and 13th, 14th or 15th dose) were log-transformed and then compared by one-way repeated measures analysis of variance with all pairwise multiple comparison procedure (Student–Newman–Keul's method) [27].

The individual pharmacokinetic analysis included the data sets of 17 patients. Due to pre-analytical problems with sampling and documentation, the data from two patients and the data from day 4 of one patient had to be excluded.

The pharmacokinetic analysis was performed using the software TOPFIT (version 2.0) [28]. The pharmacokinetic parameters were calculated using compartmental models (one and two compartments). The decline of the plasma concentrations of busulfan was best characterized by a one-compartment model with a weighting factor of $1/C^2_{obs}$. Model validation was done using the *F*-ratio-test. The data were summarized as geometric means (geometric MV) and geometric standard deviations (geometric SD), and the results measured on day 1 and 4 were compared by paired *t*-tests of the log-transformed data.

Results

Apart from oncological patients, patients with congenital metabolic diseases and immundeficiencies were included into the trial when high-dose chemotherapy with busulfan was indicated. Due to neurological symptoms, one study patient stopped treatment prematurely after the fourth dose of i.v. busulfan. Twelve patients received a dose of 0.8 mg/kg, five patients a dose of 1 mg/kg and two patients a dose of 30 mg/m^2.

The patients' demographics are given in Table 1. The age ranged from 0.9 to 17.3 years with a mean of 7.1 years (median 4.0 years). Information on body weight, height, BSA, and weight/BSA quotient are also given in Table 1.

An interim analysis done in September 2001 after accrual of five patients showed that the AUC of only two patients was outside the target range of 1000–2200 µM·min. Thus, criteria for early discontinuation of the study (more than

Table 1 Demographics of the study patients

Patient	Diagnosis	Sex	Body weight (kg)	Height (cm)	BSA (m^2)	Age (years)	Weight/BSA (kg/m^2)	Toxicity
1	AML	F	15.1	101	0.7	3.9	21.6	seizure on day 1; sepsis death on day 60
2	M. Farber	F	12.2	94	0.6	3.9	20.3	
3	AML	F	11.6	86	0.5	1.4	23.2	
4	AML	F	52	173	1.6	16.1	32.5	
5	osteopetrosis	M	29	130	1	9.2	29.0	bronchiolitis obliterans death day 280
6	MDS	F	28.4	146	1.1	12.9	25.8	
7	Ewing sarcoma	M	49.8	163	1.5	14.9	33.2	
8	JMML	F	13.8	90	0.6	2.5	23.0	respiratory failure death day 180
9	myelofibrosis	M	43.5	161	1.4	12.7	31.1	
10	Ewing sarcoma	M	25	122	0.9	7.9	27.8	agitation, aggression day 1
11	AML	M	11.3	85	0.5	1.7	22.6	
12	JMML	M	8.7	80	0.4	0.9	21.8	
13	ALL	M	10.8	81	0.5	1.4	21.6	
14	M. Hurler	F	12	76	0.5	1.8	24.0	
15	AML	M	19.2	119	0.8	7.6	24.0	
16	M. Farber	M	13.5	108	0.6	3.7	22.5	
17	MDS	M	31	145	1.1	11	28.2	
18	JMML	M	18.1	109	0.8	4	22.6	
19	AML	M	74.2	189	2	17.3	37.1	
Range		–	8.7–74.2	76–189	0.4–2.0	0.9 –17.3	20.3–37.1	
Median		–	18.1	109	0.8	4	17.7	
CV (%)		–	71	29.5	50	78.3	18.4	

AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; JMML = juvenile myelomonocytic leukemia.

two patients outside the AUC target range) were not fulfilled.

The evaluation of safety and toxicity of the test drug included the data sets of all 19 patients. Three patients died 60, 140 and 280 days, respectively, after i.v. busulfan administration. One child died due to underlying Gram-negative sepsis, the second one because of respiratory failure following terminal blast crisis and the third one after multiple organ failure following bronchiolitis obliterans organizing pneumonia.

Two patients had neurotoxic symptoms. One of these two experienced a 1-min self-limiting tonicoclonic with a rise of body temperature (above 39°C) seizure approximately 21 h after the last busulfan dose with anticonvulsive clonazepam prophylaxis during therapy. Due to a familial predisposition to convulsions during fever, the seizure was regarded as a non-serious adverse event. The other patient showed agitation, aggression and psychotic behavior (CTC grade 3) when the therapy with busulfan was started. Symptoms aggravated with changing states of consciousness, aggression, ataxia, wide responsive pupils and psychotic behavior (CTC grade 4) 1 day later. Those symptoms, although milder, were already present prior to the administration of busulfan, beginning with the first prophylactic clonazepam dosage. The infusion had to be stopped after the fourth dose, and the patient received lorazepam, phenobarbital and ketamine/midazolam.

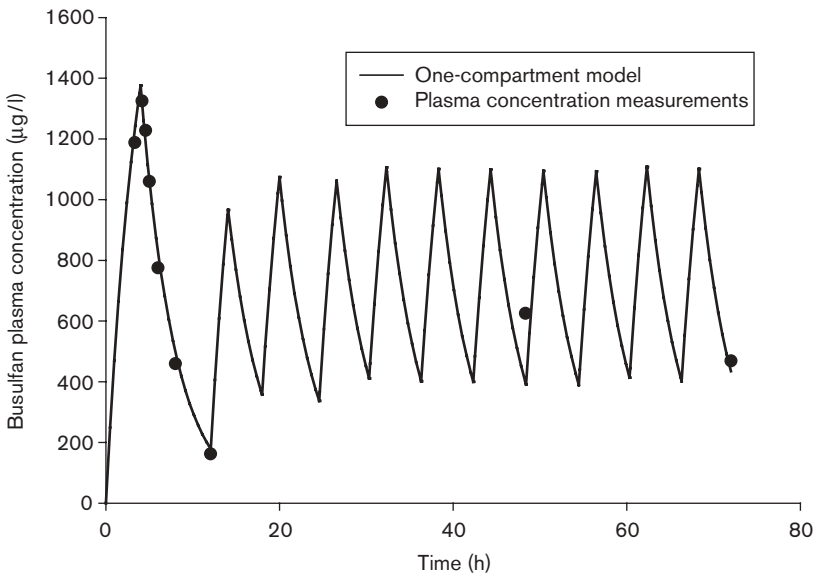
For formal reasons, the event was rated as a serious adverse event. The possible relationship with the test drug was deemed likely. The patient was free of neurological symptoms 5 days after onset and the MRT of the brain some days later was normal.

In the pharmacokinetic analysis, addition of a second compartment did not clearly improve the fit. Figure 1 shows the measured plasma concentrations and the model for a representative patient.

The dead volume in the infusion system was taken into consideration by introducing a lag time into the pharmacokinetic model, with values of up to 0.25 h allowed. The pharmacokinetic parameters are summed up in Table 2. Clearance and volume of distribution are divided by the actual body weight. The statistical comparison of the log-transformed pharmacokinetic parameters AUC, Cl, V, k_e and $t_{1/2}$ after the first dose and the last dose (day 1 versus day 4) was done by paired t-tests. Before comparing the AUC between the first administration on day 1 and the last administration on day 4, the AUC of day 1 was divided by 2 taking the doubled dose into account and assuming linear kinetics in this dosing range. There was no statistically significant difference in the parameters of day 1 and day 4 for any of the parameters.

Figure 2 shows the patients' AUC on day 1 ($n = 17$) and day 4 ($n = 9$). The AUC after the first dose ranged from 570 to 1410 μM·min (geometric mean = 1010 μM·min, geometric CV = 22%) and the AUC after the last dose ranged from 910 to 1800 μM·min (geometric mean = 1190 μM·min, geometric CV = 25%). Combining the AUCs from day 1 and 4, a geometric mean of 1070 μM·min with a geometric CV of 24% ($n = 26$) was

Fig. 1

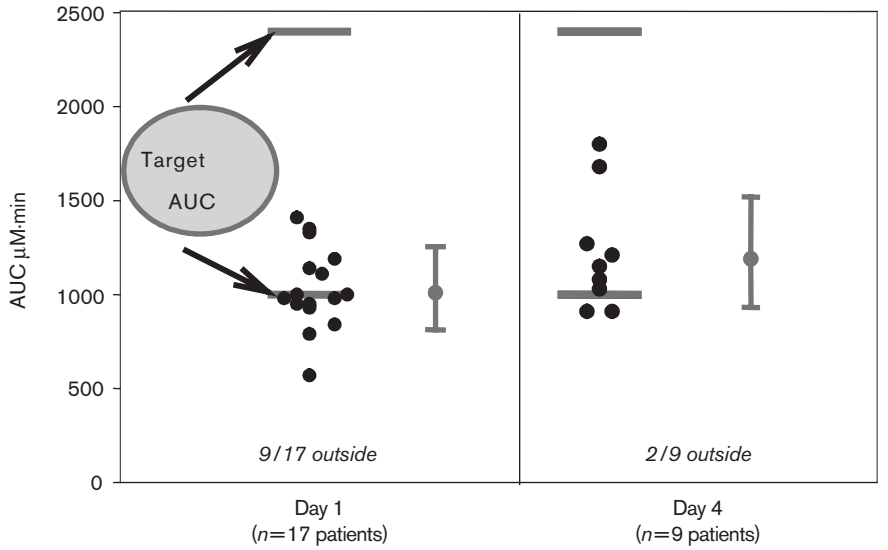


Representative busulfan concentration–time curve for one patient (ID1)

Table 2 Pharmacokinetic parameters on day 1 (first administration) and day 4 (last administration of busulfan)

	Cl (ml/min/kg)		V (l/kg)		k_e (h^{-1})		$t_{1/2}$ (h)	
	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4
N	17	9	17	9	17	9	17	9
Min	2.37	1.85	0.51	0.58	0.191	0.172	1.72	1.93
Max	5.78	3.82	1.05	0.82	0.403	0.359	3.64	4.03
Geometric mean	3.53	2.94	0.72	0.68	0.295	0.258	2.35	2.69
Geometric CV (%)	26.1	25.2	15.7	13.4	18.1	24.2	18.1	24.2

Fig. 2



Comparison of the AUC day 1 (first administration) and day 4 (last administration of busulfan). The data from the first administration were divided by 2 in order to better compare the administrations.

calculated in this patient group. In nine out of 17 patients, the AUC after the first dose was out of the target range (1000–2400 µM·min). In two out of nine patients, the AUC was outside after the last dose. In all cases, the values were below the target AUC.

Trough levels at all three time points (prior to fourth, eighth and 13th, 14th or 15th dose) were determined in nine patients and the trough levels at two time points (prior eighth and 13th, 14th or 15th dose) determined in 15 patients. The geometric mean and the geometric CV of the trough level measurements are given in Table 3. The statistical comparison of the trough levels prior to the fourth versus the eighth versus the 13th, 14th or 15th dose by analysis of variance showed no statistically significant difference ($p = 0.134$, power = 0.231, $n = 9$).

In contrast, the statistical comparison of trough levels prior to the eighth versus the 13th, 14th or 15th dose by paired *t*-tests showed a statistically significant difference ($p < 0.05$, power = 0.637, $n = 15$). The trough levels prior to the 13th, 14th or 15th were significantly lower than those prior to the eighth dose.

Discussion

Toxicity

Intravenous busulfan was studied in the context of overlapping toxicity profiles of busulfan and the excipient DMA as both substances display neurotoxic and hepatotoxic effects. Therefore, neurotoxic as well as hepatotoxic symptoms were expected.

Regarding hepatotoxicity in the 19 study patients treated with the test drug, there was no case of hepatic VOD according to the criteria of McDonald *et al.* [29].

There was one self-limiting fever-associated seizure, which was not attributed to the test drug considering the familial predisposition to convulsions during fever. A second case of neurotoxicity, in the form of psychotic behavior, agitation and aggression, was seen during the i.v.

Table 3 Trough levels prior to the fourth, eighth and 13th, 14th or 15th dose of busulfan

Patient	Trough level prior to fourth dose (μg/l)	Trough level prior to eighth dose (μg/l)	Trough level prior to 13th, 14th or 15th dose (μg/l)
1	ND	625	469
2	ND	594	448
3	443	571	477
4	932	996	981
5	414	485	412
6	659	643	227
7	ND	844	838
8	276	350	282
9	329	416	503
10[a]	–	–	–
11	408	552	452
12	ND	460	365
13	ND	474	520
14	ND	416	376
15	ND	ND	ND
16	559	531	462
17	654	658	511
18	ND	ND	ND
19	ND	ND	ND
N	9	15	15
Range	276–932	350–996	227–981
Geometric mean	486	554	459
Geometric CV (%)	40	28	37

ND = not done, no sample taken.
[a]Blood samples were discarded due to incorrect sample handling.

administration and attributed to the test drug by the local investigator.

There were no unexpected toxic effects under i.v. busulfan in our patient group ($n = 19$). Due to the small number of patients, reliable estimates of drug safety are of course not possible. Careful clinical observations of neuro- and hepatotoxicity after i.v. busulfan to children will be mandatory.

Target AUC of 1600 ± 600 μM·min

Figure 2 depicts the AUC on day 1 and day 4 of i.v. busulfan treatment. In nine out of 17 patients the AUC after the first dose was out of the target range and after the last dose the AUC in two out of nine patients was outside. Intravenous administration of 80% of the oral dose according to the protocol produced levels below the target AUC in 11 out of 26 monitored busulfan infusions (i.e. 42%).

The doses according to body weight or BSA were 0.8 mg/kg ($n = 12$), 1 mg/kg ($n = 5$) and 30 mg/m^2 ($n = 2$). Of those patients with AUC outside the target AUC after one or more busulfan doses ($n = 10$), the median age was 8.4 years (range 0.9–14.9 years). Four out of those 10 patients were younger than 2.5 years. In three of those four patients younger than 2.5 years the dosage had already been adapted to age and was increased to 1 mg/kg for i.v. treatment.

These results indicate that i.v. administration of 80% of the oral dose in children will not produce a dose intensity comparable to oral treatment. It appears that a dose escalation up to 100% of the standard oral dose in children appears to be justified in a controlled clinical study. Data on the bioavailability of oral busulfan are rare. In a crossover study with eight adults and eight children, Hassan *et al.* [30] calculated a bioavailability between 52 and 120%.

Variability of the AUC

Combining the AUC values of the first and the last dose of the i.v. treatment, the AUC ranged from 570 to 1800 μM·min (geometric mean = 1070 μM·min, geometric CV = 24%, $n = 26$). The variability of the AUC after i.v. administration (geometric CV = 24%; $n = 26$) is thus clearly lower than the variability after oral administration (geometric CV = 36%; CV = 37%; $n = 27$) according to Vassal *et al.* [10].

Two recent investigations using population pharmacokinetic methods [31,32] showed that the variability of the plasma concentrations in children after oral administration of busulfan is mainly due to deviations in the absorption rate (k_a) with CVs higher than 100%. However, both studies had the limitation that the bioavailability could not be measured. Replacing oral busulfan by i.v. busulfan in children thus appears to lead to reproducible dose intensities (as measured by AUC) with reduced variability.

Comparison of the pharmacokinetic parameters with other studies on i.v. busulfan in children

The median AUC on days 1 and 4 determined in this study is comparable to the AUC values reported by Wall *et al.* [33] in seven children (age 3–15 years) at a dosage of 0.8 mg/kg i.v. busulfan. The median AUC reported by Wall *et al.* was 909 μM·min (range 450–1306 μM·min) after the first dose and 1169 μM·min (range 885–1508 μM·min) after the ninth dose. The variation of the AUC in our study is also comparable to that reported by Wall *et al.*

Comparing our findings with those reported by Cremers *et al.* [34] after the administration of 0.8 mg/kg i.v. busulfan (age range 1.5–14 years), the geometric mean of our study (1070 μM·min, $n = 26$) clearly exceeds the geometric mean of the AUC (687 μM·min, $n = 6$) reported by Cremers *et al.* As well as the 0.8 mg/kg dose, however, our study also employed higher doses in seven patients (12 patients: 0.8 mg/kg; five patients: 1 mg/kg; two patients: 30 mg/m^2).

The geometric means of the clearance (Table 2) in this study are lower than the geometric mean [4.79 ml/min/kg (day 1)] reported by Cremers *et al.* [34].

Recently, Ngyen *et al.* published a population pharmacokinetic analysis in 24 children suggesting a dosing on body weight in four discrete categories with the highest dose of 1.2 mg/kg for patients between 9 and 16 kg [35]. However, with the age and weight distribution in our study we could not confirm the findings reported by the authors.

In our study, no statistically significant difference in the pharmacokinetic parameters AUC, Cl, V, k_e and $t_{1/2}$ between the first and the last dose of i.v. busulfan was found. However, the power of these results is small because of the limited number of study subjects. There was no reason to doubt the linear kinetics of i.v. busulfan and there seems to be no accumulation of busulfan over the 4 days of therapy.

Conclusion

Our results suggest that i.v. busulfan in children displays a smaller interpatient variability in systemic exposure compared to oral busulfan (CV of 24% after i.v. busulfan versus CV of 37% after oral busulfan). No unique toxicities were noted, although the potentially toxic DMA was used as a solvent.

The i.v. administration of 80% of the oral dose in children will not fully produce a dose intensity comparable to oral treatment of 1000–2400 μM·min. Overall, in 11 out of 26 patients, the measured AUC was below the target AUC. Further studies including a higher number of children are needed to confirm our findings.

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