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Relationship of plasma pharmacokinetics of high-dose oral busulfan to the outcome of allogeneic bone marrow transplantation in children with thalassemia

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Summary:

We analyzed plasma pharmacokinetics of busulfan in 64 children and young adults (age 2.8–26; median 11 years) with homozygous β-thalassemia transplanted with bone marrow from HLA-identical sibling donors. A uniform conditioning regimen was employed, using busulfan 14 or 16 mg/kg in 12 divided doses, and cyclophosphamide 120 or 200 mg/kg. Three sets of parameters were examined in this homogenous patient population: (1) factors that affect the plasma kinetics of busulfan, such as age and pre-transplant liver status defined by liver function tests, ferritin levels and liver biopsy; (2) busulfan-related toxicity: occurrence of veno-occlusive disease, seizures and idiopathic interstitial pneumonitis; and (3) the relationship between busulfan exposure and transplant outcome: engraftment delay or rejection, aplasia, occurrence of mixed chimeras and mortality. Kinetic analysis of first and 10th dose (using area under the curve (AUC), maximum and minimum concentration) as comparable, showing no sign of accumulation or decline in busulfan plasma levels over time. Age and liver status did not influence busulfan metabolism. No relationship was found between busulfan exposure and toxicities or transplant outcome. We conclude that busulfan monitoring is not predictive in children and young adults with homozygous β-thalassemia receiving busulfan and high-dose cyclophosphamide along with histocompatable sibling donor marrow.

Keywords: bone marrow transplantation; busulfan; pharmacokinetics; thalassemia

Busulfan is a fundamental part of myeloablative regimens used for patients undergoing bone marrow transplantation for malignant and non-malignant conditions. High-dose busulfan in combination with cyclophosphamide (CY) is particularly attractive in children, as significant total body irradiation-related mortality and morbidity, especially involving the developing central nervous system, endocrine system and skeletal system, can be avoided.

Busulfan pharmacokinetics have been studied since Ehrsson and Hassan[1] developed a gas chromatographic assay for plasma in 1983. In a preliminary study we have shown previously that variations in plasma pharmacokinetics may be age-dependent, with children younger than 5 years having a shorter elimination half-life than adults.[2] Several other studies reported the relationship between high busulfan plasma levels and drug-related toxicities, namely hepatic veno-occlusive disease (VOD) and seizures.[3,4] An inverse correlation was seen between steady-state busulfan plasma concentration and graft rejection in unrelated donor BMT in patients with multiple diagnosis.[5]

However, all previous studies were conducted analyzing heterogeneous patient populations. There has been no study in a large, uniform group of patients with a single disease, without pre-transplant chemotherapy.

We analyzed plasma pharmacokinetics of busulfan in 64 children and young adults with homozygous β-thalassemia transplanted with histocompatable sibling bone marrow. A uniform conditioning regimen was employed, using busulfan 14 or 16 mg/kg and CY 120 or 200 mg/kg. Busulfan clearance in relation to age and pre-transplant liver status was assessed and the relationship of busulfan exposure to engraftment, graft rejection and toxicities was studied.

Materials and methods

Patient population

From March 1990 to June 1991, 64 children and young adults with homozygous β-thalassemia underwent bone marrow transplantation from genotypically HLA-identical sibling donors at the Center for Bone Marrow Transplantation of Muraglia, Pesaro, Italy. Patient ages ranged from 2.8 to 26 years (median 11 years). Eleven patients (17%) were older than 18 years of age. Conditioning consisted of busulfan 14 mg/kg and CY 200 mg/kg (50 mg/kg/dose × 4 daily doses) with CsA alone as GVHD prophylaxis (regimen A) or busulfan 14 or 16 mg/kg, CY 120 mg/kg (60 mg/kg/dose × 2 daily doses) with CsA, CY (7.5 mg/kg on day +1) short-course MTX (10 mg/m^2 on days +3 and +6) with or without ALG as GVHD prophylaxis (regimen

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B). The conditioning regimen was selected based on the patient's disease class. Degree of hepatomegaly (>2 cm below the costal margin), presence of portal fibrosis and quality of chelation during the years before transplant were used to divide patients into three classes according to the absence of all risk factors (class 1), the presence of one or two factors (class 2), or the presence of all three (class 3). Patients in class 1 and 2 were prepared for transplantation using regimen A, and patients in class 3 were given regimen B[6,7] (Table 1). Bone marrow infused on day 0 contained $1.1–8.1 \times 10^8$ cells/kg (mean ± s.d. = $3.4 \pm 1.3 \times 10^8$).

Anticonvulsant prophylaxis during busulfan administration included clonazepam (56 patients), phenytoin (three patients), phenytoin and clonazepam (four patients), clonazepam and phenobarbital (one patient who was on phenobarbital for several years prior to the transplant).

All patients underwent liver biopsy before transplantation. Grading systems were established to record siderosis, chronic active hepatitis, chronic persistent hepatitis, and portal fibrosis as seen on liver biopsy. Three grades of severity (mild, moderate and severe) were identified for each diagnostic category.[8] Plasma ferritin level, SGOT and total bilirubin were also obtained pre-BMT.

Busulfan was administered orally as a tablets over 4 days every 8 h. In some instances the tablets were crushed and suspended in water at a concentration of 2 mg/ml. The actual individual dose for pharmacokinetic analysis ranged from 1.00 to 1.85 mg/kg (mean ± s.d. = 1.2 ± 0.12, median 1.17). Over the course of therapy, individual doses were adjusted to give a total dose of 14 or 16 mg/kg, and thus were not identical. Data from patients who vomited following busulfan administration were not included in the analysis.

Specimen collection and stability

Patient specimens were collected for analysis at the following times after the first and 10th dose: 0, 30, 60, 90, 120, 180, 360 min. The first dose pharmacokinetics were analyzed for all patients. Specimens for analysis of the 10th dose were obtained from 47 patients. Specimens were collected in EDTA, spun, and the plasma removed and frozen for transport. Studies have shown busulfan is stable for over 3 months in frozen plasma. Busulfan pharmacokinetics studies were performed at the University of Minnesota, Minneapolis.

HPLC assay for busulfan

A modification of the previously described HPLC assay for busulfan was used for specimen analysis.[9] Standard curves, run with each batch of patient samples analyzed, were used to quantitate busulfan. Busulfan (kindly supplied by Burroughs-Welcome, Research Triangle Park, NC, USA) was dissolved in ethyl acetate at a concentration of 75 μm. To generate busulfan standard curves the stock solution was further diluted in plasma to the following concentrations prior to analysis: 0.5, 1.0, 2.5, 5.0, 10 μm.

The standard curve and patient specimens were deproteinized and the residue obtained with diethyl-dithiocarbamic acid trihydrate (DDTC; Aldrich Chemical Co, Milwaukee, WI, USA) prior to HPLC analysis. The DDTC solution was made weekly by washing DDTC with 50 ml of ethyl acetate and adding 2.5 g to 50 ml of distilled water (5%). The DDTC solution was demonstrated to be stable for longer than 1 week. One milliliter of methanol was added to 0.5 ml of plasma, vortexed briefly and incubated for 20 min at –20°C. Tubes were spun, 0.6 ml of the supernatant was transferred to glass screw-top tubes and 0.6 ml of 100 mm ammonium acetate buffer and 0.3 ml of 5% DDTC solution was added. After mixing, the residue was allowed to stand at room temperature for 10 min. The busulfan-DDTC derivative was then extracted by adding 1.5 ml of ethyl acetate, vortexing and spun for 3 min (Sorval GLC-2, 3000 r.p.m.). One milliliter of the top (ethyl acetate) layer was transferred to glass tubes and dried at room temperature in nitrogen. The residue was resuspended in 200 μl of methanol and 100 μl injected via an autoinjector for analysis (Gilson model 231; Gilson Medical Electronics, Middleton, WI, USA). The mobile phase used was 80% methanol/20% water by volume at a flow rate of 1.5 ml/min. Absorbency at 251 nm was monitored with a UV detector (Gilson model 115) set at a sensitivity of 0.01 AUFS. The Busulfan-DDTC derivative was typically eluted at 14 min following injection. The slope of a composite of all standard curves was used to calculate the busulfan concentration in the unknown patient specimens.

Pharmacokinetic modeling

Using a personal computer, pharmacokinetic parameters were estimated by an iterative program to fit a first-order one-compartment model (MK Model; Biosoft, Milltown, NJ, USA). The following parameters were estimated from the raw data using the model: clearance (Cl, l/min/kg); volume of distribution (VD, l/kg); and absorption rate constant

Table 1 Conditioning regimen based on class of disease

Class	*No. of patients*	*Busulfan (mg/kg)*	*CY (mg/kg)*	*GVHD prophylaxis*
1–2	36	14	200	CsA
3	17	14	120	CsA + short MTX modified + ALG
3	6	16	120	CsA + short MTX modified + ALG
3	5	16	120	CsA + short MTX modified

Short MTX modified = MTX 10 mg/m^2 on days +3 and +6 and CY 7.5 mg/kg on day +1.

(Ka). The following parameters are derived from these estimates: absorption half-life (ab $t_{1/2}$, min) and elimination half-life (el $t_{1/2}$, h). Estimates of the plasma concentrations for each time-point are derived from Cl, VD and Ka. The following parameters were determined from the derived plasma concentration-time curve: area under the concentration-time curve (AUC, μm*min); maximum concentration (Cmax, μm); and minimum concentration (Cmin, μM). For direct comparisons between groups, the AUC, Cmax and Cmin were normalized to a dose of 1 mg/kg by dividing the actual value by the dose administered. In a minority of patients (less than 10%) pharmacokinetic modeling could not be done because an initial estimate of the hybrid elimination rate constant could not be generated from the fall-off data.

Outcome parameters

Evaluation of regimen-related toxicities included VOD, seizures, and idiopathic interstitial pneumonitis. VOD was defined clinically by the presence of hepatomegaly, weight gain and increase in bilirubin.[10] The diagnosis of idiopathic interstitial pneumonitis was made on a radiograph when an infectious agent was not found.

Post-transplant engraftment was determined by an increase in the granulocyte count (ANC $>5\times10^8$/l), cytogenetic analysis when the donor and recipient were of opposite sex and globin chain synthesis evaluation by column chromatography. Graft rejection was defined as a loss of documented donor engraftment with return of autologous marrow. Occurrence of mixed chimerism was evaluated by DNA study at 60–120 days post-BMT.[11]

Acute and chronic graft-versus-host disease was graded according to the Seattle criteria.[12]

Statistical analysis

Three sets of parameters were examined: factors that might affect the pharmacokinetics of busulfan (eg age, pre-transplant liver function tests, ferritin levels); possible drug-related toxicity (eg VOD, seizures, interstitial pneumonitis), and transplant outcome (eg aplasia, engraftment delay or rejection, mixed chimerism, and mortality). *t*-test, ANOVA, and multiple linear regression were employed in evaluating predictors of busulfan pharmacokinetics, and in assessing the association of busulfan pharmacokinetics with toxicity. Kaplan–Meier product limit method and Cox regression analysis were applied to evaluate the effect of busulfan pharmacokinetics on time to engraftment and survival, censoring patients at death for engraftment and last contact for survival.

Results

Pharmacokinetic parameters

Pharmacokinetic parameters normalized to a busulfan dose of 1 mg/kg are summarized in Table 2 for the entire patient group. Due to the small dose range there was insignificant change in AUC, Cmax, Cmin following dose normalization to 1 mg/kg.

Table 2 Busulfan pharmacokinetic summary

Parameter	*Median*	*Mean ± s.d.*	*Range*
AUC_F	650	700 ± 390	180–2900
AUC_L	740	900 ± 520	240–2900
Cmax_F	2.7	3.0 ± 1.1	1.0–5.4
Cmax_L	3.4	3.8 ± 1.4	1.4–7.6
Cmin_F	0.45	0.54 ± 0.36	0–1.9
Cmin_L	0.54	0.65 ± 0.52	0–2.2
CL_F	6.1	7.1 ± 3.6	1.4–21
CL_L	5.2	5.5 ± 2.5	1.6–15
VD_F	0.86	0.96 ± 0.43	0.38–2.7
VD_L	0.69	0.76 ± 0.34	0.24–2.0
AB_F	22	26 ± 17	0–78
AB_L	21	25 ± 20	0–87
EL_F	1.6	1.7 ± 0.9	0.6–7.0
EL_L	1.5	1.9 ± 1.6	0.4–9.1

AUC = area under curve normalized to a dose of 1 mg/kg (μm*min). To convert to an average steady-state concentration (ng/ml) multiply by 0.683; Cmax = maximum concentration (μm); Cmin = minimum (trough concentration) (μm); CL = clearance (ml/min/kg); VD = volume of distribution (l/kg); AB = absorption half-time (min); EL = elimination half-time (h); __F = 'first' dose; __L = 'tenth dose'.

Comparison of first and 10th dose kinetics revealed that they are similar, being within 20% of each other the majority (90%) of the time. For AUC, Cmax, CL, VD there is a significant difference in the distribution between first and last dose, but the mean difference is small (<10%) and therefore probably not of biological significance. The differences between the first and 10th dose are of small magnitude (<20%) for the most part, suggesting that there is no need to determine both. The data indicate no busulfan accumulation or decrease in exposure with subsequent doses due to changes in metabolism, as has been reported with phenytoin.

There is no significant association between pharmacokinetic parameters and age. The mean clearance of busulfan in our study was approximately twice that reported in adults and similar to values previously reported in young children.[2] Analysis comparing busulfan pharmacokinetics in children 5 years of age and younger ($n=7$) with patients older than 5 years ($n=57$) showed no statistical difference.

Patient pre-BMT clinical status was characterized by results of liver biopsy, SGOT, total bilirubin, ferritin and assigned a disease class, summarized in Tables 3 and 4. There is a significant correlation between ferritin level and

Table 3 Pre-transplant liver status – biopsy results

No. of patients	*None*	*Mild*	*Moderate*	*Severe*
Siderosis	0	15	28	20
Fibrosis	9	14	24	16
CAH	41	8	12	1
CPH	36	26	0	0

CAH = chronic active hepatitis; CPH = chronic persistent hepatitis. Biopsies from two patients were not evaluable for hepatitis and biopsy from one patient was not evaluable for siderosis and fibrosis.

Table 4 Pre-transplant liver status – liver function tests

	Median	*Mean ± s.d.*	*Range*
Bilirubin (mg/dl)	1.0	1.4 ± 1.1	0.2–7.1
SGOT (mU/ml)	23	28 ± 17	5–103
Ferritin (ng/ml)	2476	3019 ± 1823	710–9017

disease class, degree of siderosis, degree of fibrosis and SGOT ($P = 0.0005$, $P = 0.0001$, $P = 0.0001$, $P = 0.0001$, respectively). However, there is no correlation between pre-transplant liver status or the class and busulfan pharmacokinetics.

Busulfan concentration and outcome

Marrow recovery (ANC $>0.5 \times 10^9$/l) occurred on days 13–37, with a medium of 18 days. Busulfan exposure, CY dose and type of GVHD prophylaxis did not affect time to neutrophil recovery.

The influence of busulfan on the incidence of graft rejection (five rejections were observed, a total of 7.5%) was not evident. No difference in pharmacokinetic parameters was observed for patients who rejected their graft as compared to those with sustained engraftment. However, only six patients had average steady-state concentrations below 200 ng/ml, the range of increased rejection for HLA matches reported by Slattery *et al*,[5] and none rejected. Rejection was associated with CY dose (5/27 rejected when treated with 120 mg/kg *vs* 0/30 with 200 mg/kg, $P = 0.02$) and GVHD prophylaxis (5/23 rejection noted with CsA/ALG/MTX *vs* 0/36 with CsA, $P = 0.02$). As the patients treated with CY 120 mg/kg also received CsA/ALG/MTX it is impossible to identify the effect of CY and GVHD prophylaxis on graft rejection.

There was no significant relationship between busulfan exposure and late aplasia, which occurred in five patients (7.5%), or the incidence of mixed chimerism (27%, on an analysis conducted on days 60–180). The mortality rate was 15%. In univariate analysis older age (<15 years *vs* >15 years), Cmax, higher ferritin levels and occurrence of GVHD were significantly related to mortality, while in multiple regression analysis only GVHD occurrence significantly correlated with a higher rate of mortality ($P < 0.01$).

Busulfan concentration and toxicities

In our patient population the incidence of VOD was 4.5% (three patients), interstitial pneumonitis 11% (seven patients), early onset seizures 7% (five episodes before day +30 post-BMT). No seizures during administration of busulfan were documented. Busulfan plasma level was not associated with these events although only one patient had an AUC previously associated by Grochow with a higher risk of VOD.[3] Two patients diagnosed with VOD received CY doses of 200 mg/kg, and one received a CY dose of 120 mg/kg. VOD was significantly ($P < 0.006$) associated with ferritin (Figure 1) levels as well as with degree of siderosis on liver biopsy. Three of 20 patients with severe siderosis were diagnosed with VOD, in contrast to 0 of 43 with mild or moderate siderosis ($P = 0.02$).

To eliminate the potential influence of different CY doses and GVHD prophylaxis regimens on transplant outcome and toxicities, additional analyses were conducted on patients with class 1 and 2 disease. There was no significant relationship between busulfan exposure and transplant outcome in the 36 patients who received busulfan 14 mg/kg, CY 200 mg/kg and CsA only as a GVHD prophylaxis.

Discussion

Our study was conducted on a homogenous patient population transplanted with bone marrow from HLA-matched genotypically identical siblings. In this group there was no association of busulfan pharmacokinetics with age or with

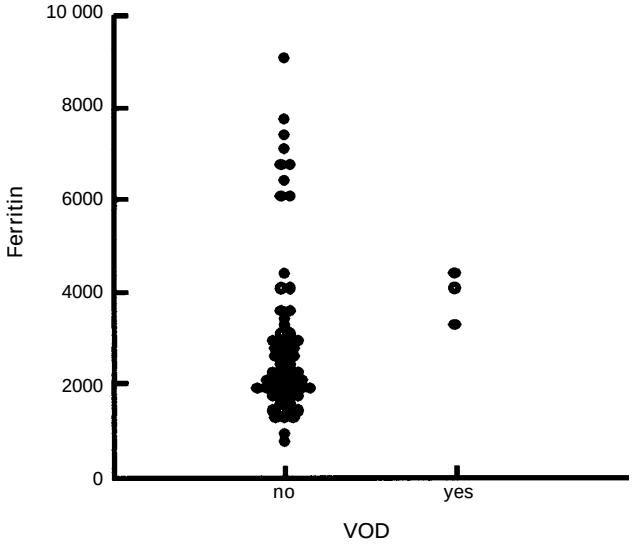

Figure 1 Ferritin (ng/ml) and hepatic veno-occlusive disease (VOD).

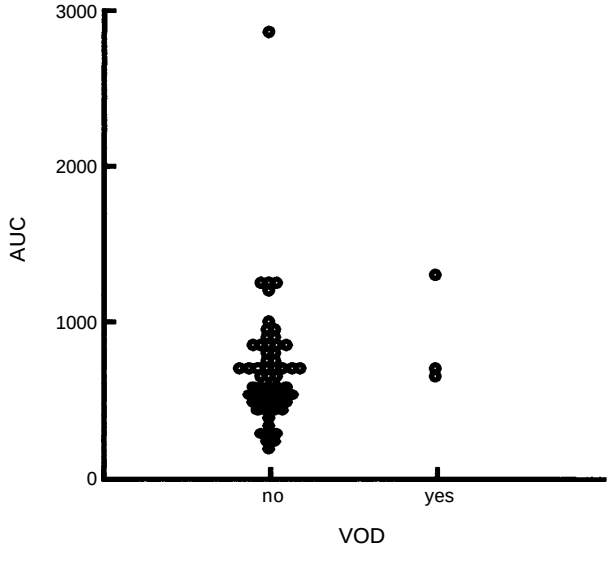

Figure 2 Busulfan exposure (AUC; μm/min) and hepatic veno-occlusive disease (VOD).

pre-transplant liver status. We demonstrated a similarity between kinetics of first and last busulfan doses. No correlation between busulfan exposure and engraftment, graft rejection, late aplasia, occurrence of mixed chimerism, incidence of VOD, seizure, interstitial pneumonitis, or death was observed.

Analysis conducted in such a homogenous group of patients can ensure a more accurate assessment of regimen-related toxicities because of the uniformity of the pre-transplant treatment regimen and clinical status. Such an analysis allows us to correlate busulfan pharmacokinetics more directly with treatment outcome.

Initial evaluation of low-dose oral busulfan in adults (2, 4 and 6 mg dose) by Ehrsson *et al*[13] was followed by studies of high-dose busulfan (1 mg/kg) conducted by the same group. Analysis of first and last dose pharmacokinetics showed no evidence of busulfan accumulation over time. Subsequent studies had variable results with one being confirmatory[4] and others showing either increases or decreases in busulfan levels occurring with consecutive doses.[2,14] For example, a decrease in busulfan plasma levels over the 4 day treatment period has been reported by Hassan *et al*[15] in about 40% of adults and children, suggesting that busulfan can induce its own metabolism. However, recently this alteration in busulfan plasma level was attributed to concomitant administration of phenytoin used as seizure prophylaxis. Phenytoin and phenobarbital have been shown to decrease the lethal effects of a myeloablative dose of busulfan in mice[16] suggesting that simultaneous administration of drugs that alter liver metabolism can affect busulfan kinetics. In our study, patients received anticonvulsant prophylaxis although most received clonazepam and not phenytoin or phenobarbital. Kinetics of first and last busulfan doses were similar, showing no evidence of accumulation or decrease in plasma levels over time. We conclude that measurement of first-dose kinetics is sufficient for assessment of busulfan exposure during BMT conditioning in children and young adults with homozygous β-thalassemia if there are no changes in subsequent busulfan doses.

Variations in plasma pharmacokinetics have been described to be age dependent. Grochow *et al*[2] reported that children between 2 months and 5 years demonstrated differences in busulfan disposition when compared with adults.[2] Hassan *et al*[17] found children younger than 5 years to have a shorter busulfan elimination half live. In our series, which included children and young adults, there is no association between age and busulfan pharmacokinetics. It suggests that in the context of BMT for thalassemia there is no need for adjustment of busulfan dose based on body surface area in children which has been reported to be associated with increased extrahematological toxicities.[18]

We also failed to show an association between busulfan clearance and pre-transplant liver status. In our patient population with a wide spectrum of liver damage (documented by liver biopsy) secondary to multiple transfusions and hepatitis there is no evidence of busulfan clearance being affected by liver function. Other studies have shown that pre-existing liver disease with elevated AST and ALT are significant pre-transplant risk factors for developing VOD regardless of the conditioning regimen.[19] We observed that the degree of siderosis on liver biopsy and ferritin levels, which reflected severity of disease, were significantly associated with occurrence of VOD. However, we failed to demonstrate a correlation of AUC values with VOD and other toxicities. Grochow *et al*[3] originally correlated VOD with increased mean busulfan AUC ($>$2012 μm*min). A later prospective study by Grochow *et al*[20] confirmed the increased occurrence of VOD with AUC $<$1500 μm*min. They also noted a significant decrease in incidence of VOD in patients receiving reduced doses of busulfan from the 5th to the 16th doses as compared to patients without dose reduction. However, Schuler *et al*[21] were unable to correlate busulfan pharmacokinetics parameters with the risk of VOD in adults with CML receiving HLA-identical BMT, a disease with decreased risk of VOD. Also, a single daily dose regimen (4 mg/kg/dose), which resulted in a four-fold higher Cmax and AUC values (dose adjusted) was not associated with an increase in VOD incidence.[22] A trial conducted by Demirer *et al*[23] attempted to decrease the variability of busulfan plasma concentration by busulfan dose adjustment based on first dose pharmacokinetics. Targeted plasma levels were reliably achieved with dose adjustment. Comparison with previous experience showed no improvement in VOD occurrence or BMT outcome. However, as more conditioning agents are added to the regimen it becomes difficult to obtain relationships between outcome and exposure to any one of the agents. Dix *et al*[24] were also able to achieve the desired busulfan plasma levels with dose adjustment without affecting VOD frequency. As dose adjustments were made very late, reduction in toxicity may not have been seen.

The incidence of VOD in our study was low – 4.5%. There was no correlation with plasma busulfan exposure and VOD (Figure 2). Only one patient had busulfan plasma level above the critical concentration obtained by Grochow *et al*,[3] 1500 μm*min (2855 μm*min) and he did not develop VOD. We conclude that in younger thalassemia patients undergoing HLA-identical BMT monitoring of plasma busulfan level does not have predictive value for the development of VOD.

A syndrome that occurs rarely and usually only after long-term or high-dose busulfan therapy is known as 'busulfan lung'. Interstitial pneumonitis with lethal outcome was initially reported in a high proportion of patients receiving conditioning doses of busulfan/CY. However, subsequent studies have shown an incidence similar to or less than that observed after CY with total body irradiation.[25] In our study, busulfan exposure did not influence occurrence of pneumonitis.

Seizures have been reported during treatment with high-dose busulfan at any time from the second dose up to 24 h following the final dose.[4] We did not observe seizures during this time period although all patients received anticonvulsant prophylaxis. The occurrence of seizures later following BMT were not related to the busulfan pharmacokinetic parameters analyzed.

Our results suggest that graft rejection following preparation with conventional doses of busulfan and CY does not correlate with busulfan concentration. Slattery *et al*[5] demonstrated low steady-state busulfan concentrations (Css $<$200 ng/ml) in patients rejecting their graft after partially HLA-matched sibling or matched unrelated donor BMT.

However, in the HLA-matched sibling grafts, busulfan concentration was a much less important determinant of rejection. Those results were confirmed by Regazzi *et al*[26] who did not observe graft rejection in any of eight transplants from HLA-identical sibling donors who had Css >200 ng/ml. In our study of transplants from HLA-matched siblings, rejection was associated with a lower CY dose (120 mg/kg) and type of GVHD prophylaxis (CsA, ATG, MTX), but not plasma busulfan plasma exposure. As patients conditioned with 120 mg/kg CY also received CsA, ATG, MTX it is impossible to separate the influence of these two variables on graft rejection. However, in the patient population with β-thalassemia analyzed by Lucarelli *et al*[7] conditioning with high-dose CY (200 mg/kg *vs* <200 mg/kg) was associated with a significantly lower rejection rate, regardless of GVHD prophylaxis.

We found no association between busulfan concentration and post-transplant mortality. Survival was decreased only by occurrence of GVHD.

In summary, we can conclude that busulfan pharmacokinetics are not predictive in children and young adults with homozygous β-thalassemia receiving busulfan/CY and bone marrow from a matched sibling. Further studies are needed to define the role of busulfan pharmacokinetics in patients undergoing unrelated or mismatched BMT and in very young or older patients undergoing HLA-matched transplants.

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References

1 Ehrsson H, Hassan M. Determination of busulfan in plasma by gas chromatography with selected ion monitoring. *J Pharm Sci* 1983; **72**: 1203–1205.
2 Grochow LB, Krivit W, Withley CD, Blazar B. Busulfan disposition in children. *Blood* 1990; **75**: 1723–1727.
3 Grochow LB, Jones RJ, Brundrett RB *et al*. Pharmacokinetics of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. *Cancer Chemother Pharmacol* 1989; **25**: 55–61.
4 Vassal G, Gouyette A, Hartman O *et al*. Pharmacokinetics of high-dose busulfan in children. *Cancer Chemother Pharmacol* 1989; **24**: 386–390.
5 Slattery JT, Sanders JE, Buckner CD *et al*. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant* 1995; **16**: 31–42.
6 Lucarelli G, Galimberti M, Polchi P *et al*. Bone marrow transplantation in patients with thalassemia. *New Engl J Med* 1990; **332**: 417–421.
7 Lucarelli G, Clift RA, Galimberti M *et al*. Marrow transplantation for patients with thalassemia: results in class 3 patients. *Blood* 1996; **87**: 2082–2088.
8 Muretto P, Del Fiasco S, Angelluci E *et al*. Bone marrow transplantation in thalassemia: modification of hepatic iron overload and associated lesions after long-term engrafting. *Liver* 1994; **14**: 14–24.
9 Henner WD, Furlong EA, Flahetry MD, Shea TC. Measurement of busulfan by high-performance liquid chromatography. *J Chromatogr* 1987; **416**: 426–432.
10 McDonald GB, Sharma P, Matthews DE *et al*. Venocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. *Hepatology* 1984; **4**: 116–122.
11 Andreani M, Manna M, Lucarelli G *et al*. Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. *Blood* 1996; **87**: 3494–3499.
12 Glucksberg H, Storb R, Fefer A *et al*. Clinical manifestation of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donor. *Transplantation* 1974; **18**: 295–304.
13 Ehrsson H, Hassan M, Ehrnebo M, Beran M. Busulfan kinetics. *Clin Pharmacol Ther* 1983; **34**: 86–89.
14 Yeager AM, Wagner JE Jr, Graham ML *et al*. Optimization of busulfan dose in children undergoing bone marrow transplantation: a pharmacokinetic study of dose escalation. *Blood* 1992; **80**: 2425–2428.
15 Hassan M, Ehrsson H, Smedmyr B *et al*. Cerebrospinal fluid and plasma concentration of busulfan during high dose therapy. *Bone Marrow Transplant* 1989; **4**: 113–114.
16 Fitzsimmons WE, Ghalie R, Kaizer H. The effect of hepatic enzyme inducers on busulfan neurotoxicity and myelotoxicity. *Cancer Chemother Pharmacol* 1990; **27**: 226–228.
17 Hassan M, Oberg G, Bekassy AN *et al*. Pharmacokinetics of high-dose busulfan in relation to age and chronopharmacology. *Cancer Chemother Pharmacol* 1991; **28**: 130–134.
18 Vassal G, Deroussent A, Challine D *et al*. Is 600 mg/m^2 the appropriate dosage of busulfan in children undergoing bone marrow transplantation? *Blood* 1992; **79**: 2475–2479.
19 Locasciulli A, Bacigalupo A, Alberti A *et al*. Predictability before transplant of hepatic complications following allogeneic bone marrow transplantation. *Transplantation* 1989; **48**: 68–72.
20 Grochow LB. Busulfan disposition: the role of therapeutic monitoring in bone marrow transplantation induction regimen. *Semin Oncol* 1993; **20** (Suppl. 4): 18–25.
21 Schuler U, Schroer S, Kuhnle A *et al*. Busulfan pharmacokinetics in bone marrow transplant patients: is drug monitoring warranted? *Bone Marrow Transplant* 1994; **14**: 759–765.
22 Shaw PJ, Scharping CE, Brian RJ Earl JW. Busulfan pharmacokinetics utilizing a single daily dose regiment in children with acute leukemia. *Blood* 1994; **84**: 2357–2362.
23 Demirer T, Buckner CD, Appelbaum FR *et al*. Busulfan, cyclophosphamide and fractionated total body irradiation for allogeneic marrow transplantation in advanced acute and chronic myelogenous leukemia: phase I dose escalation of busulfan based on targeted plasma levels. *Bone Marrow Transplant* 1996; **17**: 341–346.
24 Dix SP, Wingard JR, Mullins RE *et al*. Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant* 1996; **17**: 225–230.
25 Van der Jagt RHC, Appelbaum FR, Petersen FB *et al*. Busulfan and cyclophosphamide as a preparative regimen for bone marrow transplantation in patients with prior chest chemotherapy. *Bone Marrow Transplant* 1991; **8**: 211–215.
26 Regazzi MB, Locatelli F, Buggia I *et al*. Disposition of high dose busulfan in pediatric patients undergoing bone marrow transplant. *Clin Pharmacol Ther* 1993; **54**: 45–52.