Treosulfan pharmacokinetics and dynamics in pediatric allogeneic stem cell transplantation

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HIGH INTERPATIENT VARIABILITY OF TREOSULFAN EXPOSURE IS ASSOCIATED WITH EARLY TOXICITY IN PAEDIATRIC HSCT: A PROSPECTIVE MULTICENTRE STUDY

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ABSTRACT

Treosulfan-based conditioning is increasingly employed in paediatric hematopoietic stem cell transplantation (HSCT). Data on treosulfan pharmacokinetics in children are scarce, and the relationship between treosulfan exposure, toxicity and clinical outcome is unresolved. In this multicentre prospective observational study, we studied treosulfan pharmacokinetics and the relation with regimen-related toxicity and early clinical outcome in 77 paediatric patients. Treosulfan dose was 30 g/m^2, administered over 3 consecutive days in infants <1 year old (n=12), and 42 g/m^2, in children ≥1 year old (n=65), respectively. Mean day 1 treosulfan exposure was 1,744±795 mg*hr/L (10 g/m^2) and 1,561±511 mg*hr/L (14 g/m^2), with an inter-individual variability of 56 and 33%, respectively. High treosulfan exposure (>1,650 mg*hr/L) was associated with an increased risk of mucosal (OR 4.40; 95%CI 1.19-16.28, *P*=.026) and skin toxicity (OR 4.51; 95%CI 1.07-18.93, *P*=.040). No correlation was found between treosulfan exposure and the early clinical outcome parameters engraftment, acute graft-versus-host disease, and donor chimerism. Our study provides the first evidence in a large cohort of paediatric patients for high variability in treosulfan pharmacokinetics and an association between treosulfan exposure and early toxicity. Ongoing studies will reveal whether treosulfan exposure is related to long-term disease-specific outcome and late treatment-related toxicity.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for a variety of malignant and non-malignant inherited or acquired diseases. The conditioning regimen given prior to HSCT has two goals: suppression of the immune system of the host to prevent graft rejection and allow donor engraftment, and ablation of dysfunctional or malignant host hematopoietic cells. Based on differences in underlying diseases, patient characteristics including age and co-morbidity, and HSCT strategy, various conditioning regimens, which differ in immuno- and myeloablative potential, have been developed. Myeloablative regimens based on total body irradiation (TBI) or high exposure busulfan are effective in facilitating engraftment and disease control, but share the disadvantage of being associated with significant early and late toxicity [1-3]. One of the major challenges to improve HSCT is reducing toxicity caused by the conditioning regimen given while maintaining efficacy. Treosulfan is an alkylating agent that is increasingly employed as part of conditioning regimens in HSCT for both malignant and non-malignant diseases due to its favourable toxicity profile in comparison to busulfan and TBI.

Treosulfan (L-threitol 1,4-bismethanesulphonate, Ovastat®) is a prodrug and a water-soluble alkylating agent. It is non-enzymatically, pH-dependently converted into a monoepoxide- and a diepoxide-derivative, which are thought to be responsible for DNA alkylation, interstrand DNA crosslinking, chromosomal aberration and induction of apoptosis [4, 5]. Treosulfan has potent myeloablative potential that is comparable with that of busulfan [6]. Furthermore, the immunosuppressive profile of treosulfan has been demonstrated to be stronger in comparison to that of busulfan and more durable than that of cyclophosphamide [6]. An *in vitro* study has shown that treosulfan has a stronger cytotoxic effect against paediatric leukemic cells compared to busulfan [7]. Recently, several studies have reported efficacy and tolerability of treosulfan-based conditioning regimens in paediatric HSCT for both non-malignant and malignant diseases [8-15].

Studies have shown that busulfan has highly variable pharmacokinetics, and clinical outcome of HSCT using busulfan-based conditioning is dependent on busulfan

exposure. Therefore, therapeutic drug monitoring (TDM) is required to achieve optimal drug exposure in individual patients [16, 17]. Similarly, we assume that clinical outcome with respect to both toxicity and efficacy after HSCT after a treosulfan-based regimen might also be dependent on actual drug exposure. To date, only a few studies, including small numbers of paediatric patients, investigated the pharmacokinetic profile of treosulfan. These studies focused on pharmacokinetics (PK) and reported substantial interpatient variability up to 70%. However, the association between treosulfan exposure and clinical outcome parameters was not addressed in these studies [18-21].

In this report of a multicentre prospective study, we describe the pharmacokinetics of treosulfan in paediatric HSCT recipients and the relationship between treosulfan exposure and early toxicity and clinical outcome.

PATIENTS AND METHODS

Characteristics of the study cohort

A total of 77 paediatric patients transplanted between June 2011 and July 2016 who received conditioning with treosulfan and fludarabine (TF) or treosulfan, fludarabine and thiotepa (TFT) prior to HSCT in the Leiden University Medical Center in The Netherlands and the Children's Hospital Bambino Gesù in Rome, Italy were included in this study. The institutional Ethics Committee approved the treosulfan PK study protocol (P12.267). Written informed consent for participation in the study was obtained from the parents or legal guardians, as well as consent from patients when they were older than 12 years according to the Helsinski Declaration (last amended in 2013, Fortaleza Brasil).

Patient and donor characteristics are summarized in Table 1. Forty-six (59.7%) patients were males and 31 (40.3%) females. The median age in our cohort at transplantation was 4.8 (IQR 1.6-11.4) years, 12 patients were <1 years old. Patients received HSCT for various malignant and non-malignant indications in line with the

EBMT–Working Party Inborn Errors and Paediatric Diseases recommendations and based on previous reports on treosulfan-based conditioning [9, 10, 13]. Most patients (84.4%) were transplanted for a non-malignant disease, including 31 (40.3%) patients with hemoglobinopathy, 22 (28.5%) patients with primary immune deficiency, 11 (14.3%) with an inherited bone marrow failure syndrome and 1 (1.3%) patient with a metabolic disease. Patients with malignant disease received treosulfan as part of a reduced toxicity regimen because of pre-existent co-morbidity. Seventy-three (94.8%) patients received treosulfan-based conditioning preceding a first HSCT, whereas in four patients the drug was used in preparation to a second transplantation. Sixty-five patients (84.4%) above the age of 1 year received treosulfan in a total dose of 42 g/m^2, administered over 3 consecutive days (14 g/m^2 per day). Twelve patients (15.6%) under the age of 1 year old received a total dose of 30 g/m^2 (10 g/m^2 per day). Treosulfan was combined with thiotepa (8-10 mg/kg) and fludarabine in 52 patients (67.5%) (total dose of 150-160 mg/m^2), whereas in 25 patients (32.5%) treosulfan was combined with fludarabine. Thiotepa was administered at day -8, treosulfan at day -7 to day -5 and fludarabine at day -7 to day -3. Serotherapy consisted of anti T lymphocyte globulin (ATLG), anti thymocyte globulin (ATG) or alemtuzumab. Twenty-seven patients (35.0%) received a transplant from a HLA identical sibling, 36 patients (46.8%) from a matched unrelated donor (≥9/10 allelic matching) and 14 patients from a HLA-haploidentical relative (18.2%). In patients with a mismatched related donor, peripheral blood stem cell grafts were processed by either CD34-positive selection or selective elimination of αβ+ T and CD19+ B cells [22]. Graft-versus-Host Disease (GvHD) prophylaxis was given according to institutional guidelines. Granulocyte colony-stimulating factor (G-CSF) was routinely given in cord blood transplants from day +8 onwards. All patients were cared for in high-efficiency, particle-free air (HEPA)-filtered positive-pressure isolation rooms and received intestinal decontamination using non-absorbable antimicrobials, as well as supportive care according to institutional guidelines.

Table 1. Patient characteristics

	Total (n=77)
Characteristic	
Age (years, IQR (median))	1.6-11.4 (4.8)
Weight (kg, IQR (median))	10.8-34.2 (17.0)
Sex (n: M/F)	46/31
Diagnosis for HSCT	
Hemoglobinopathies (%)	31 (40.3)
Hematologic malignancy (%)	12 (15.6)
Primary immune deficiency (%)	22 (28.5)
Bone marrow failure (%)	11 (14.3)
Other (%)	1 (1.3)
Number of transplantation	
First (%)	73 (94.8)
Second (%)	4 (5.2)
Donor	
MSD (%)	27 (35.0)
MUD (≥ 9/10) (%)	36 (46.8)
MMFD (haplo) (%)	14 (18.2)
Stem cell source	
BM (%)	50 (64.9)
PBSC (%)	20 (26.0)
T cell replete (%)	6 (7.8)
TCR αβ/CD19 depletion (%)	11 (14.3)
CD34 enrichment (%)	3 (3.9)
CB (%)	6 (7.8)
BM + CB (%)	1 (1.3)
Conditioning	
Treo-Flu-Thiotepa (%)	52 (67.5)
Treo-Flu (%)	25 (32.5)
Treosulfan dose	
14 g/m^2 (%)	65 (84.4)
10 g/m^2 (%)	12 (15.6)
Serotherapy	
Yes (%)	69 (89.6)
ATG (%)	38 (49.3)
ATLG (%)	22 (28.6)
Alemtuzumab (%)	9 (11.7)
No (%)	8 (10.4)
GvHD prophylaxis	
CsA (%)	4 (5.2)
CsA / MTX (%)	50 (64.9)
CsA / Pred (%)	3 (3.9)
Other (%)	6 (7.8)
None (%)	14 (18.2)

BM: bone marrow, PBSC: peripheral blood stem cells, CB: cord blood, MSD: matched sibling donor, MMFD: mismatched family donor, MUD: matched unrelated donor, Treo: treosulfan, Flu: fludarabine, Thio: thiotepa, ATG: Anti thymocyte globulin, ATLG: Anti T lymphocyte globulin, GvHD: Graft-versus-Host Disease, CsA: Cyclosporine A, MTX: methotrexate, Pred: prednisolone.

Treosulfan assay

Blood samples were collected in serum tubes without gel. Samples were centrifuged as soon as possible (within 5 hours), and stored at -20 degrees Celsius. A validated reversed-phase high-pressure liquid chromatography (RP-HPLC) using ultraviolet (UV) detection was used to determine treosulfan concentration in serum, as previously reported [21]. Briefly, treosulfan and the internal standard busulfan were made detectable through derivatization with sodium diethyldithiocarbamate (DDTC). Linearity was established up to 500 mg/L with a lower limit of quantification of 6.8 mg/L. Accuracies of quality control (QC) samples were within the 90-110% limit. The intraday imprecision, expressed as coefficient of variation (CV%), ranged from 2.0% to 3.3% and interday imprecision ranged from 2.1% to 2.8%.

Population pharmacokinetics of treosulfan

The individual pharmacokinetic parameters of each patient were determined using a validated two-compartment population pharmacokinetic (PK) model, using non-linear mixed-effects modelling as implemented in the NONMEM software package (version 7 level 3; Icon Development Solutions, Ellicott City, Maryland, USA). This is an extended model based on the PK model published by Ten Brink et al [21]. A total of 384 samples were used in a range of 1.5, 3.5, 4, 5, 7 and 9 hours after the start of a 3-hour infusion to develop and validate the model. Clearance and volume of distribution were allometrically scaled using body weight. The structural PK model of treosulfan indicated the following PK parameters for a child with 20 kg body weight: average clearance (CL) of 6.98 L/h (CV: 37.9%), average central distribution volume (Vc) of 9.59 L (CV: 54.2%), average peripheral distribution volume (Vp) of 2.34 L (CV: 82.6%) and average inter-compartmental clearance of 2.74 L/h (CV: 69.1%). The allometric scaling exponent for clearance was fixed at 0.75 and for volume of distribution at 1.0. AUCs were calculated using post-hoc estimation using the final model based on all available samples. In 29 patients rich curves were obtained and used. Using the limited sampling strategy established by Ten Brink et al. and

reconfirmed with the extended model as described above, 2 samples were taken in the following 48 patients at 4 and 7 hours after start infusion to calculate AUC [21]. For determination of the intra-patient variability blood samples obtained on day 3 in a subgroup of patients (n = 19) were also used in the analysis.

Evaluation of clinical data

Primary endpoint of this study was early toxicity and secondary endpoints were early clinical outcome parameters (i.e. engraftment, acute GvHD and chimerism at day +30 and +100). Toxicity endpoints were evaluated until 28 days after HSCT and included mucosal, skin, hepatic and neurological toxicity measured according to CTCAE criteria and Bearman et al [23]. Engraftment was defined as the first of three days with a neutrophil count of $\geq 0.5 \times 10^9$/L. Platelet engraftment was defined as platelet count $> 50 \times 10^9$/L, without platelet support for 3 consecutive days. Chimerism was determined in peripheral blood granulocytes and mononuclear cells by VNTR polymorphism at day 30 and 100 after transplantation. Acute GvHD was diagnosed and graded as defined by Przepiorka et al [24].

Statistical analysis

Normally distributed parameters are shown as mean ± standard deviation and all log-normally distributed parameters as median (interquartile range IQR). Inter-patient variability was calculated by the coefficient of variation (CV%) of the treosulfan exposure between individuals and intra-patient variability by calculating the mean difference between the AUC on day 1 and day 3 of each individual. The predictive value of systemic treosulfan exposure for the occurrence of toxicity within 28 days is evaluated using a logistic regression analysis for mucosal, skin, hepatic and neurological toxicity events. Cumulative toxicity was scored as the sum of these different toxicities, with a maximum score of 4 and tested as two groups (≥ 2 toxicities, yes/no). This is tested with AUC as a discrete variable, considering 3 exposure groups low [<1350 mg*hr/L (1st tertile)], medium [1350-1650 mg*hr/L (2nd tertile)] and high [>1650

mg*hr/L (3rd tertile)]. The cumulative incidence of neutrophil engraftment and acute GvHD was estimated using the method of Fine and Gray for censored data subject to competing risks, taking into account graft failure, death without engraftment and subsequent HSCT as competing risk for neutrophil engraftment and death before day +100 as competing risk for acute GvHD [25]. The association between treosulfan exposure and acute GvHD and engraftment is tested with the Gray test using two AUC groups with the median as cut-off value (1500 mg*hr/L). The relationship between treosulfan exposure and chimerism at day +30, +100 and 1 year after HSCT was determined with ordinal logistic regression, using 3 groups (donor chimerism <50%, 50-90% and >90%) and AUC as a continuous variable. All P-values were 2-tailed and considered significant when $P < .05$. Statistical analyses were performed with SPSS statistics, version 23.0 (IBM Corp., Armonk, NY, USA). The competing risks analysis and Gray test was performed using R version 3.4.0.

RESULTS

Treosulfan pharmacokinetics

A total of 96 AUCs were determined in 77 patients. The results are shown in Figure 1 and Table 2. In the first 19 patients treosulfan AUC was determined on day 1 and day 3 to assess intra-patient variability. The mean intra-patient variability was 13.9% (Figure 2). Based on these results we decided to determine treosulfan exposure only on day 1 as a good representation of total exposure.
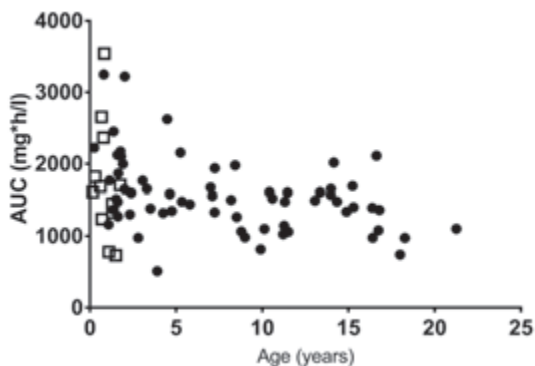
Figure 1. The relation between treosulfan exposure and age. The vertical axis represents the Area under the Curve (AUC) values in mg*hr/L. Symbols represent the different dosing schemes: 14 g/m² (●), 10 g/m² (□).

Table 2. Treosulfan pharmacokinetic parameters

Parameter	10 g/m² mean ± SD	14 g/m² mean ± SD
No. of patients	12	65
Age (yrs)	0.9 ±0.48	7.96 ± 5.75
Weight (kg)	7.4 ±1.9	27.59 ± 18.15
BSA (m²)	0.37 ± 0.07	0.94 ± 0.41
AUC (mg*hr/L)	1744 ± 795	1561 ± 511
Cl (ml/min/kg)	2.17 ± 1.41	8.08 ± 5.04
Clp (ml/min/kg)	1.61 ± 0.46	3.00 ± 2.44
Vc (L/kg)	0.14 ± 0.09	0.74 ± 0.63
Vp (L/kg)	0.07 ± 0.04	0.17 ± 0.15

BSA: body surface area, AUC: area under the curve, Cl: clearance, Clp: inter-compartmental clearance, Vc: central volume of distribution, Vp: peripheral volume of distribution

The mean day 1 exposure was 1,561 mg*hr/L (range 511-3,250 mg*hr/L) and 1,744 mg*hr/L (range 732-3,544 mg*hr/L) for patients that had received 14 g/m² and 10 g/m², respectively (P = .263). The corresponding inter-patient variability (CV%) was 33% and 56% within the groups that had received 14 g/m² and 10 g/m², respectively, showing large inter-patient variability of treosulfan exposure, especially in young children. Because of dose adjustment to 10 g/m² in young children, mean exposure

did not significantly differ, however mean clearance was significantly lower in children receiving 10 g/m^2 compared to 14 g/m^2 (2.17 vs. 8.08 ml/min/kg, P < .001) and mean central volume of distribution was also lower (0.14 vs 0.74 L/kg, P < .001).
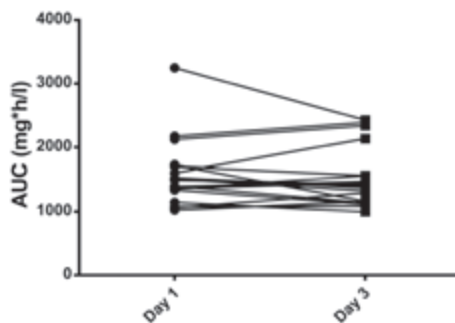
Figure 2. Intra-patient variability of treosulfan. AUC was measured at day 1 and day 3 in 19 patients (13.9%).

Early regimen-related toxicity

Early regimen-related toxicity was evaluated until 28 days after HSCT. The most common toxicities observed were mucosal-, hepatic-, skin- and neurological toxicity.

Mucositis. Thirty-six (46.8%) patients experienced mucositis, with 25 patients developing grade 2 (19.5%) or 3 (13.0%). Grade 4 mucositis was not seen in any of the patients. The occurrence of mucositis in the different exposure groups is shown in Figure 3. The odds of developing grade 2 or greater mucositis was significantly higher when AUC exceeded 1,650 mg*hr/L (OR 4.40; 95% CI 1.19-16.28, P = .026) compared to AUC under 1,350 mg*hr/L. Given the fact that there were two different conditioning regimens (TF and TFT), we corrected for this covariate together with age. The adjusted OR was 7.03 (95% CI 1.60-30.86, P = .010). A higher risk to develop grade 2-3 mucositis is also seen in the medium AUC group (1350-1650) compared to AUC under 1350 mg*hr/L, however this did not reach statistical significance (adjusted OR 3.66; 95% CI 0.93-14.52, P = .065). Accordingly, higher treosulfan AUC is associated with the risk of higher grade mucositis (P = .006) (Figure 4).
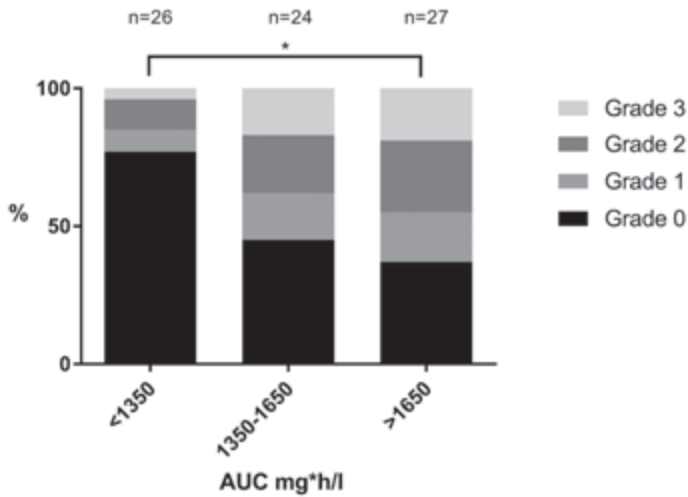
Figure 3. Incidence of mucositis in different treosulfan exposure groups. The incidence of mucositis is shown according to grade, with black being grade 0, progressing to light gray being grade 3. (*Grade 0/1 vs grade 2/3, *P* = .026).
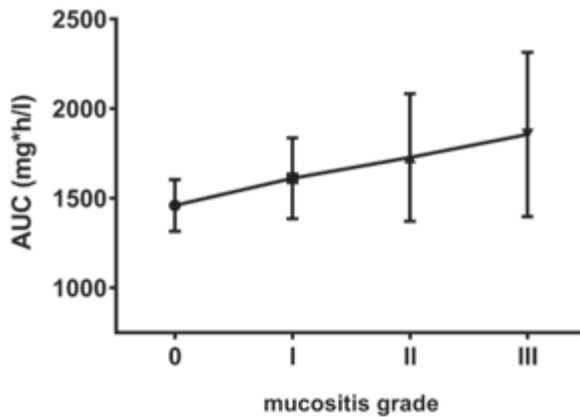

Figure 4. Mean treosulfan AUC with 95% CI according to mucositis grade. The vertical axis represents the (AUC) values in mg*hr/L with 95% confidence interval. Treosulfan AUC is associated with mucositis grade (*P* = .006).

Hepatic toxicity. Hepatic toxicity grade 2 or greater, defined as a >5-fold increase in alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) levels and more than a 3-fold increase in bilirubin levels, was seen in 24 patients (31.2%). There is no statistically significant association between treosulfan exposure and hepatic toxicity. The odds of developing grade 2 or greater hepatic toxicity are possibly influenced by treosulfan exposure, but no statistically significant association was seen (Table 3). Severe veno-occlusive disease/sinusoidal obstruction syndrome, according to modified Seattle criteria, was not seen in any of the patients [26, 27].

Skin toxicity. Skin toxicity, which includes erythematous rash and skin exfoliation, occurred in 18 (23.2%) patients (Figure 5). Children belonging to the high AUC group (>1,650 mg*hr/L) showed an increased risk of developing skin toxicity (OR 4.51; 95% CI 1.07-18.93, *P* = .040 and OR 9.96; 95% CI 1.85-53.46, *P* = .007, adjusted for conditioning regimen and age). An increased risk was also seen in the 1,350-1,650 mg*hr/L group, although the difference with the low exposure group is not statistically significant (Table 3). The addition of thiotepa to the conditioning regimen also showed a trend of increased risk of skin toxicity, however this was not statistically significant.
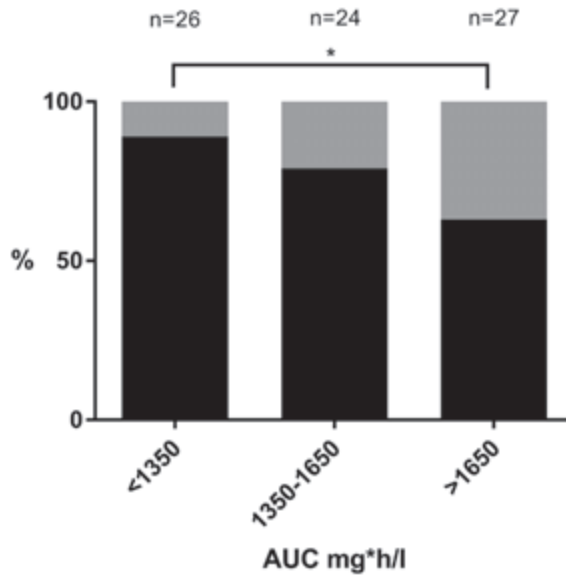
Figure 5. Incidence of skin toxicity in different treosulfan exposure groups. Skin toxicity: gray, no skin toxicity: black (*P = .040).

Neurological toxicity. Five patients (6.5%) experienced neurological symptoms, including convulsions and posterior reversible encephalopathy syndrome (PRES). No significant association was seen between neurological symptoms and treosulfan exposure (Table 3).

Cumulative toxicity. We investigated whether AUC is correlated with the occurrence of multiple toxicities including mucositis, skin and/or liver toxicity. Of all patients, 46.8% experienced no toxicity, 20.8% experienced one toxicity, 23.4% two toxicities and 9.1% experienced three toxicities. The risk of experiencing two or more toxicities is higher when AUC exceeds 1650 mg*hr/L compared to AUC under 1350 mg*hr/L (OR 4.52; 95% CI 1.32-15.53, P = .016 and OR 8.25; 95% CI 1.88-36.13, P = .005, adjusted for conditioning regimen and age).

Table 3. Relationship between treosulfan AUC and early regimen-related toxicity

Treosulfan AUC mg*hr/L	Mucositis	p	Hepatic toxicity	p	Skin toxicity	p	Neurological toxicity	p	Cumulative toxicity ≥ 2 toxicities	p
<1350										
OR (95%CI)	Reference		Reference		Reference		Reference		Reference	
Adjusted OR (95%CI)										
1350-1650										
OR (95%CI)	3.30 (0.86-12.71)	.083	1.12 (0.33-3.84)	.860	2.02 (0.43-9.55)	.376	-		1.40 (0.37-5.37)	.624
Adjusted OR (95%CI)	3.66 (0.93-14.52)	.065	1.19 (0.34-4.22)	.786	2.38 (0.48-11.78)	.289			1.56 (0.39-6.21)	.531
>1650										
OR (95%CI)	4.40 (1.19-16.28)	.026	1.60 (0.50-5.13)	.432	4.51 (1.07-18.93)	.040	1.50 (0.23-9.80)	.672	4.52 (1.32-15.53)	.016
Adjusted OR (95%CI)	7.03 (1.60-30.86)	.010	2.40 (0.64-8.93)	.193	9.96 (1.85-53.46)	.007	1.77 (0.22-14.24)	.659	8.25 (1.89-36.13)	.005

AUC: area under the curve, OR: Odds Ratio, CI: Confidence interval. The adjusted OR was adjusted for conditioning regimen and age.

3

GvHD

Cumulative incidence of grade II-IV acute GvHD was 11% (95% CI 6-21%) with 4 patients experiencing grade II and 4 patients grade III GvHD. None of the patients developed grade IV GvHD. Within the TF group, only 1 of 22 (4.5%) evaluable patients at day +100 developed grade II-III acute GVHD, compared to 7 of 45 (15.5%) in the TFT group. Two of the four patients with grade II and all four with grade III acute GvHD were transplanted from an unrelated donor including two with a T-cell-replete peripheral blood stem cell graft. Mean treosulfan exposure in patients with and without GvHD was 1,365 mg*hr/L and 1,579 mg*hr/L, respectively. There was no significant difference between the two groups (P = .108).

Engraftment, chimerism and survival

Cumulative incidence of neutrophil engraftment was 94% (95% CI 89-100%). Median time to neutrophil and platelet engraftment was 19 (IQR 15-23) days and 22 (IQR 11-34) days, respectively. All the 7 patients experiencing graft failure, three with a T-cell replete and four with T-cell depleted graft, received a total treosulfan dose of 42 g/m^2; 5 patients were conditioned with TFT and 2 patients with TF. Mean AUC was 1,605 mg*hr/L and 1,342 mg*hr/L in the engraftment and non-engraftment group, respectively. No relationship was found between engraftment and treosulfan AUC (P = .750). Four patients with non-engraftment underwent subsequent second transplantation, whereas the other three patients died because of treatment-related complications.

There were 72 patients evaluable for day +30 chimerism, 63 patients for day +100 chimerism and 58 patients at 1 year after HSCT. In 63 patients (87.5%), 44 patients (69.8%) and 38 patients (65.5%) donor chimerism was between 90 and 100% for day +30, +100 and 1 year, respectively. No relationship was seen with treosulfan exposure (P = .857, P = .535 and P = .500 for day +30, +100 and 1 year, respectively). Cumulative incidence of treatment related mortality at day +100 was 10.4% (95% CI 5-20%) and no relationship was found with treosulfan exposure. Overall survival in this cohort was 86%. No relationship was seen with treosulfan exposure (data not shown).

3

DISCUSSION

Treosulfan-based conditioning is increasingly used in paediatric HSCT and has been demonstrated to be effective and well tolerated in patients with both malignant and non-malignant diseases [9, 10, 12, 13, 28-30]. Despite the increased use of treosulfan in recent years, pharmacokinetic data in children are still limited. To the best of our knowledge, this is the largest paediatric cohort studied so far, covering a broad age range. We demonstrate large inter-patient variability of treosulfan exposure. Furthermore, pharmacokinetic parameters were shown to be age-dependent with higher AUC values in younger children (<1 year old) and corresponding lower treosulfan clearance. This is the first study that reports the relationship between treosulfan exposure and early clinical outcome parameters. We provide evidence that higher treosulfan exposure is associated with a higher risk of drug related toxicity and specifically with moderate to severe mucositis and skin toxicity. Also, a higher exposure is a predictor for experiencing multiple toxicities.

In our study, the mean day 1 AUC of 1,561 mg*hr/L in patients receiving 14 g/m^2 was lower than that reported by Glowka et al. (2,400 ± 1,267 mg*hr/L), but slightly higher than that reported by Koyyalamudi et al. (1,412 ± 215 mg*hr/L) [18, 20]. However, those results were based on small number of patients, i.e. 7 and 3 patients, respectively (age range 2-18 years), which may contribute to explain these differences. When comparing our pharmacokinetic data with previous studies, we found an intra-patient variability of 13.9% (CV), based on measurements in 19 patients, which is much lower than the inter-patient variability (33-56%), and lower than reported by Glowka et al. [18]. In our cohort, a subgroup of 12 children <1 year old received a dose of 10 g/m^2. In these infants, treosulfan exposure was higher (mean of 1,744 mg*hr/L) compared with older patients receiving 14 g/m^2 (mean of 1,561 mg*hr/L) with an inter-patient variability of 56%. In previous studies, PK data in very young children are scarce and point to either an increased AUC or no difference in AUC compared to older children [19, 20, 31]. We found that treosulfan clearance in children under the age of 1 year, receiving 10 g/m^2, was significantly lower than that of older children receiving 14 g/m^2.

It is unclear why younger children, despite an already adjusted lower dose, have higher AUC values. A possible explanation for this phenomenon could be the maturation and development of renal function in children under the age of one year. The glomerular filtration rate increases rapidly during the first two weeks of life, but adult values are not reached until 8-12 months [32]. Because approximately 25% of treosulfan is excreted via a renal route in unchanged form, the not fully matured renal function of these young infants could be an explanation of lower treosulfan clearance [33]. Scheulen et al. hypothesized that this observation could be explained by metabolic acidosis associated with the release of large amount of methanesulfonic acid during treosulfan activation which causes inhibition of pH-dependent treosulfan transformation [34]. We believe this is unlikely to be the explanation in our patients, given the buffering action of blood and the relatively stable clinical situation of the patients prior to administration of treosulfan. Either way, the difference in AUC between infants and older children warrants further investigation. Most conditioning protocols recommend dose adjustment, i.e. 10 g/m^2 or 12 g/m^2, in children <1 years old to limit toxicity. Our PK data show that the dose adjustment in younger children results in comparable treosulfan exposure in children under and above 1 years old. Whether similar exposure as a consequence of current age-adjusted dosing leads to similar clinical outcome in these different age groups remains to be demonstrated.

Treosulfan itself is a pro-drug and converted into its active derivatives (a monoepoxide and diepoxide) in a non-enzymatic and pH-dependent manner. In this study, we focused on the PK of the parent compound, as we thought this to be a good representation of alkylating activity. Recently, the concentrations of the metabolites of treosulfan have been analyzed in patients [18]. In this study the diepoxide derivative could not be detected in patient samples, probably due to fast elimination and the monoepoxide derivative was found in concentrations approximately two-order lower that the parent compound. Whether the metabolite exposure is also in association with early toxicity is yet unclear and requires further investigation.

Our study provides evidence of a relationship between treosulfan exposure and early toxicities (in particular severe mucositis and skin toxicity), whereas we did not yet find

an association with engraftment, occurrence of severe acute GvHD and chimerism at day +30, +100 and 1 year. This may however be due to the heterogeneity of primary diseases in our cohort. PK/PD studies in more homogeneous and single disease patient cohorts with longer follow up will be of great value to study the relationship of AUC and these long-term outcome parameters. These studies are currently ongoing.

Our observation that high treosulfan exposure is associated with a higher risk of moderate to severe mucositis, skin toxicity and a higher risk of experiencing multiple toxicities in the first 28 days after SCT indicates that avoiding high exposure may reduce transplant-related morbidity in individual patients. Because of the great inter-patient variability and different pharmacokinetic parameters in very young children, therapeutic drug monitoring as a personalized approach may be a suitable option to optimise individual outcome in this group. However, before considering such an approach the relationship between treosulfan exposure and long-term disease specific outcome needs to be established as well. Also, longer follow up of these patients is needed to investigate whether the occurrence of late toxicities, particularly those involving gonadal function, is correlated with treosulfan exposure, and how this compares to other myeloablative regimens based on the use of either busulfan or irradiation.

In this study, we provide, for the first time, evidence for high variability in pharmacokinetic parameters of treosulfan in children. There is an inverse relationship between AUC and age, suggesting that the adjusted dose in very young children is justified to achieve an exposure which is similar to older children. High treosulfan exposure is associated with the occurrence of moderate to severe mucositis and skin toxicity. Ongoing studies will reveal whether treosulfan exposure is related to long-term disease outcome and late treatment-related toxicity.

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