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Chapter 2

Personalized busulfan and treosulfan conditioning for pediatric stem cell transplantation: the role of pharmacogenetics and pharmacokinetics

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ABSTRACT

Busulfan- and treosulfan-based conditionings are the cornerstone of pediatric allogeneic hematopoietic stem cell transplantation (HSCT). Although both drugs are alkylating agents, their mechanisms of action, pharmacokinetics (PK) and toxicity profiles are different. Experience with busulfan in pediatric HSCT is broad and the knowledge on the pharmacodynamics (PD), PK and, to a lesser extent, pharmacogenetics (PG) has resulted in a more effective therapy. Treosulfan has only recently been introduced in pediatric HSCT and is considered a promising new therapy because of its beneficial toxicity profile. However, knowledge of the PK and PG of treosulfan is limited. In this review, we describe the pharmacology of both agents and discuss factors causing variability in PK in relation to therapeutic outcome in HSCT.

INTRODUCTION

Allogeneic HSCT (allo-HSCT) is a potential curative treatment for a range of hematological malignancies and non-malignant diseases in pediatric patients. The main reasons for treatment failure are relapse and treatment-related mortality (TRM). TRM can occur in up to 5–20% of patients,^{1–3} mostly caused by infections, graft-versus-host disease (GvHD) or toxic organ damage, which is dependent on the underlying disease, donor matching, graft source, supportive care and conditioning regimen administered before the HSCT. A conditioning regimen usually comprises a combination of immunosuppressive and myeloablative drugs with the goal to cause suppression of the host immune system to prevent rejection, create 'space' in the bone marrow of the recipient to allow donor cell engraftment, and, if applicable, eliminate the underlying malignancy. The alkylating agents busulfan (1,4-butanediol-dimethylsulfonate, Busilvex®) and treosulfan (L-threitol 1,4-bismethanesulphonate, Ovastat®) are commonly applied in different conditioning regimens before HSCT. Both drugs have different profiles in relation to the toxicity and mechanism of action in HSCT. In this review, we describe the PD, PK and PG profiles of both drugs. The experience with busulfan-based conditioning in pediatric patients is extensive compared with that of treosulfan. In recent years, a large series of PK, PD and PG studies has reported on the optimization of busulfan treatment. We provide an overview of the most important findings and of future perspectives on how to further optimize busulfan dosing. Dose-optimization studies for treosulfan are scarce and lessons might be learned from previous studies in busulfan.

Mechanism of action of busulfan

Busulfan is a bifunctional alkylating agent of the alkylsulfonate type, comprising two instable methanesulfonate groups (Figure 2.1). Busulfan is hydrolyzed in aqueous environments and releases the methanesulfonate groups, leading to a reactive carbonium ion that alkylates DNA. Busulfan is only slightly soluble in water, although an intravenous formulation became available in 2000. It was first applied in the palliative treatment of chronic myeloid leukemia (CML) for its myelosuppressive properties and antitumor effects.⁴ Furthermore, busulfan is mainly cytotoxic for proliferating tissues and depletes non-cycling primitive stem cells. Although busulfan is a strong myelosuppressive drug, it is only weakly immunosuppressive; at a dose causing 50% decrease in myelopoiesis, only a mild decrease in lymphocyte numbers is observed.⁵

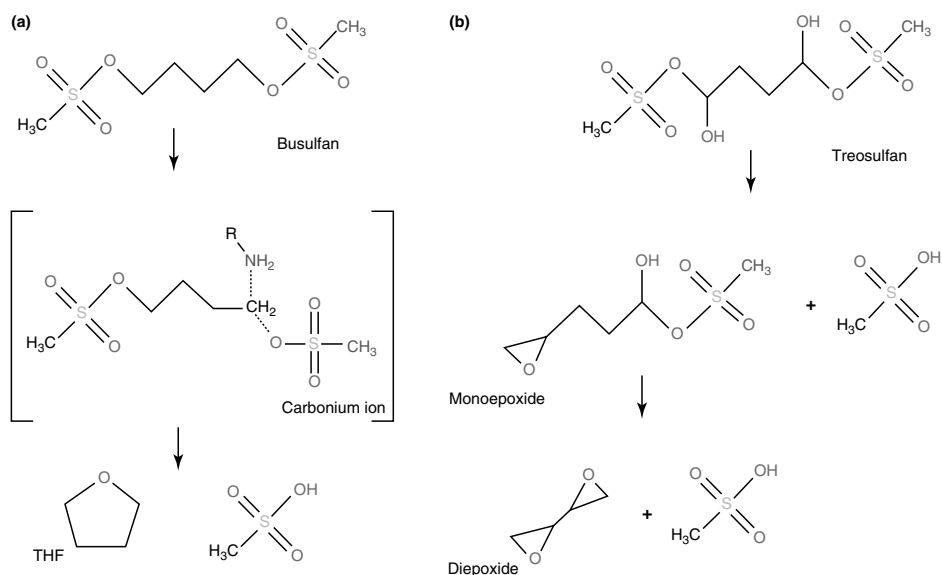


Figure 2.1 Activation of busulfan and treosulfan. a) Busulfan is hydrolysed and releases one methanesulphonate group. Through the reaction with a thiol group ($\text{NH}_2\text{-R}$) an instable carbonium ion is formed. When releasing the second methanesulphonate group, tetrahydrofuran (THF) is formed. b) Treosulfan is non-enzymatically, pH-dependently converted by intramolecular nucleophilic substitution into a monoepoxide ((2S,3S)-1,2-epoxy-3,4 butanediol 4-methanesulphonate) and a diepoxide (L-diepoxybutane).

Mechanism of action of treosulfan

Treosulfan has a strong myeloablative potential and is considered less toxic than busulfan and, therefore, an interesting alternative for busulfan in conditioning before HSCT. The first clinical application of treosulfan in pediatric patients prior to HSCT was in 2002.⁶ Treosulfan is a prodrug and a water-soluble alkylating agent. It is non-enzymatically, pH-dependently converted by intramolecular nucleophilic substitution into a monoepoxide ((2S,3S)-1,2-epoxy-3,4 butanediol 4-methanesulphonate) and a diepoxide (L-diepoxybutane) (Figure 2.1).⁷ Conversion occurs at a pH > 6.0 and the conversion to the monoepoxide is necessary for DNA alkylation, DNA crosslinking occurs via the diepoxide only.⁸ Treosulfan gives a rapid and sustained myeloablation, which is comparable to that of busulfan. This was demonstrated by a fast reduction in colony-forming unit granulocyte macrophages in mice; aplasia was reached on day 1 and was maintained after completion of treatment.⁹ Furthermore, the immunosuppressive profile of treosulfan was demonstrated by a strong and durable splenic B and T cell depletion and low pro-inflammatory cytokines release. *In vitro* and *in vivo* data suggest a stronger immunosuppressive and cytotoxic effect against leukemic cells compared with busulfan.^{9,10}

Busulfan and treosulfan in malignant disease

In malignant disease, myeloablative conditioning (MAC) is usually used, aimed at myeloablation and maximum reduction of leukemic cells. High-dose busulfan or total-body irradiations are the cornerstones of MAC. Initially, busulfan was mainly combined with cyclophosphamide, which is an effective therapy, but is accompanied by severe toxicities. Recently, several attempts have been made to maintain the efficacy of a MAC, but reduce the toxicity; for example, by targeting busulfan exposure and the replacement of cyclophosphamide with fludarabine.

In malignant disease, treosulfan-based conditioning was only recently introduced. It is usually used in patients who are not eligible for the standard preparative regimen because of pre-existent morbidity and in cases of second HSCT after initial traditional myeloablative conditioning. Casper *et al.*¹¹ initially investigated treosulfan in malignant disease in adult patients, but studies on treosulfan in malignant disease in pediatric patients are limited. A large retrospective study on behalf of the European Society for Blood and Marrow Transplant (EBMT) Pediatric Diseases Working Party on the effectiveness and safety of treosulfan-based conditioning was performed in pediatric patients with high risk or advanced hematologic malignancies.¹² The treosulfan-based conditioning demonstrated efficacy rates similar to rates found in busulfan-based studies and the toxicity profile was comparable to that of reduced intensity conditioning (RIC). Although treosulfan seems to be a promising candidate in HSCT for malignant disease, there is no direct comparison of busulfan- and treosulfan-based conditioning. Therefore, a prospective study directly investigating both agents in malignant diseases in pediatric patients is warranted.

An overview of all recent studies (2008–2013) using busulfan- and treosulfan-based conditioning in allo-HSCT in pediatric patients is provided in Tables 2.1 and 2.2, respectively. This period was selected as an update of the review by Glowka *et al.* on treosulfan.¹³

Busulfan and treosulfan in non-malignant disease

An increasing number of non-malignant disorders in pediatric patients are suitable for allo-HSCT. These patients often have severe comorbidities or are very young. In these types of disease, the main goal of HSCT is to establish normal donor hematopoiesis and to reverse or halt disease progression. The level of donor engraftment that is needed for cure is dependent on the disease and the extent of engraftment of a certain lineage of hematopoietic cells. The main risk of allo-HSCT in non-hematologic disease is graft rejection and toxicities related to the HSCT, such as acute GvHD (aGvHD), infections and organ toxicity. Busulfan-cyclophosphamide conditioning was the standard conditioning in non-malignant diseases. To reduce the toxicity

Table 2.1 Overview of recent studies and outcomes in busulfan-based conditioning before allogeneic HSCT in pediatric patients

Patients (n) and median age (range)	Underlying disease ^a	Donor matching and source ^b	Conditioning regimen ^c	Engraftment and chimerism ^d	GvHD ^e	Toxicities ^f	Survival ^g	Ref
114 4.8 yr FluBu 2.6 yr BuCy (0.2–19 yr)	Non-malign.: 39% Malignant: 61%	MSD: 18% UCB: 66% UBM: 16%	Bu/Flu: 56% Bu IV dosing on BW ⁱ AUC: 80–95 mg ^h /L Flu: 40 mg/m ² /d 4d Bu/Cy/(Mel): 44% Bu IV: 80 mg/m ² <1 yr or 120 mg/m ² > 1yr AUC: 74–82 mg ^h /L Cy: Malignant: 60 mg/kg/d 2d Non-malign.: 50 mg/kg/d 4d Mel: 140 mg/m ² /d 2d (in malignant diseases) ATG: 10 mg/kg 4d in UD	BuFlu: ANC(0.5): 17d Platelet(50): 40d BuCy: ANC(0.5): 21d Platelet(50): 56d	cGvHD: BuFlu: 9% BuCy: 26%	Lung injury: BuFlu: 16% BuCy: 36% SOS: BuFlu: 3% BuCy: 28%	OS 2yr: BuFlu: 82% BuCy: 78% EFS 2yr: BuFlu: 78% BuCy(Mel): 72%	⁷⁵
18 14 yr (10–18)	Thal M	MFD: 61% MUD: 17% MMFD: 11% MMUD: 11%	Bu/Flu/ATG Bu: 130 mg/m ² /d 4d Flu: 35 mg/m ² /d 4d ATG: 1.5 mg/kg/d 3d + sequential pretransplant immunosuppression Flu: 40 mg/m ² /d 5d Dex: 25 mg/m ² /d 5d 1 or 2 cycles	Primary engraftment: 100% ANC(0.5): 12d Plts(20): 18d 2 nd mixed chim: 11%	aGvHD: gr 2–4: 22% gr 3–4: 11% lim cGvHD: 28%	Mucositis gr 1–2: 22% Mild SOS: 16%	OS: 89% DFS: 89%	⁹¹

44	8 yr (1–21)	Thal M Cl 1: 16% (MAC) Cl 2: 55% (MAC) Cl 3: 30% (RIC)	MSD: 91% MFD: 9% BM: 73% (MAC) PBSC: 27% (RIC)	Bu/Cy/ATG (MAC): 70% Bu oral: 5 mg/kg/d 4d <3 yr or 4 mg/kg/d 4d >3 yr Cy: 50 mg/kg/d 4d ATG: 30 mg/kg/d 5d Bu/Flu/ATG/TLI (RIC): 30% Bu oral: 4 mg/kg/d 2d Flu: 35 mg/m ² /d 5d ATG: 30 mg/kg/d 5d	Primary engraftment: 100% MAC: ANC(0.5): 15 d Plts(20): 21 d RIC: ANC(0.5): 14.5 d Plts(20): 18 d 2 nd Graft failure: 11.3% MAC: 6% RIC: 23%	aGvHD≥2: 21% MAC: 16% RIC: 31% lim cGvHD: 7% MAC: 6% RIC: 8% ext cGvHD: 5% MAC: 6% RIC: 0%	Mild SOS: 20% MAC: 23% RIC: 15% Gonadal failure: MAC: 13% RIC: 31% Growth failure: ⁱⁱⁱ MAC: 13% RIC: 8%	OS 5yr: 98% MAC: 97% RIC: 100% DFS 5yr: 86% MAC: 90% RIC: 77% TRM: 2% MAC: 6% RIC: 0%	92
44	12.5 yr (4–14)	ALL CR1: 41% CR2: 32% CR3: 16%	MSD: 95% MMFD: 2% MMUD, CB: 2% BM: 9% PBSC: 91%	Bu/Cy Bu oral: 4 mg/kg/d 4d Cy: 60 mg/kg/d 2d ST: Mismatched	Primary engraftment: 100% ANC(0.5): 12d Plts(20): 15d	aGvHD: gr 1–2: 82% gr 3–4: 25% lim cGvHD: 23% ext cGvHD: 5%	No data	OS: 80% OS 3yr: 68% DFS 3yr: 50% Relapse: 34%	93
35	7 yr (0.4–20)	Non-malign.: 86% Malignant: 14% (all myeloid)	MFD: 34% MUD: 46% MMUD 9/10: 20% BM: 57% PBSC: 37% BM+CB: 3% BM+PBSC: 3%	Bu/Flu/Amab Bu IV: 3.6–4.8 mg/kg/d 4d C _{ss} : 900 ng/ml malignant C _{ss} : 600 ng/ml non-malign. Amab: 0.5mg/kg/d 3d Flu: 1.3 mg/kg/d 4d (<4yr) or 40 mg/m ² /d 4d (>4yr)	Non-engraftment: 9% ANC (0.5): 17d Median donor chimerism: 1 mnt: 99% 2 yr: 92%	aGvHD: 17% gr 1–2: 14% gr 4: 3% cGvHD 8%	Infusion reaction to Amab gr 1: 20% gr 2: 34% Mucositis gr 3–4: 43% Moderate SOS: 3% (+ prophylaxis)	OS: 78% TRM: 9% EFS: 61% Non-malign.: 66% Malignant: 40%	94

Table 2.1 continues on next page

Table 2.1 *Continued*

Patients (n) and median age (range)	Underlying disease ^a	Donor matching and source ^b	Conditioning regimen ^c	Engraftment and chimerism ^d	GvHD ^e	Toxicities ^f	Survival ^g	Ref
12 16 yr (2–20)	Malignant: 58% APML, CML, NHL, Other Non-malign.: 42%	PBSC: 75% RD: 17% UD: 58% UCB: 17% UBM: 17%	Bu/Flu/Amab Bu IV: 4 mg/kg/d 4d (≤ 4 yr) or >4yr 3.2mg/kg/d 4d C _{ss} : 600–900 ng/ml or AUC: 1800–2400 mmol*min/L Flu: 30mg/m ² /d 6d Amab: 54 mg/m ² in 5 d (max 83 mg)	ANC(0.5): 16d Platelet(20): 31d Chimerism: 1 mnt: 93% donor chim 3 mnt: 88.6% donor chim Graft failure: 8%	aGvHD: gr 2–4: 42% gr 3–4: 25% lim cGvHD: 8%	Any gr 3–4: 8% Liver gr 4: 8% Infections: Fungal: 25% Line: 50%	OS 3yr: 91%	⁹⁵
27 8.6 yr (3.3–17.4)	SCA	BM or MSD: 100%	Bu/Cy/ATG Bu oral or IV: 3.5 mg/kg/d 4d < 1500 $\mu\text{mol}^*\text{min/L}$: 40% 900–1100 $\mu\text{mol}^*\text{min/L}$: 40% 575–877 $\mu\text{mol}^*\text{min/L}$: 20% Cy: 50 mg/kg/d 4d ATG: 30 mg/kg/d 3d	Chimerism: Full donor: 84% Stable mixed: 16%	aGvHD: gr 1–2: 12% gr 3–4: 4%	SOS: 30% Pneumonitis: 4% Seizures: 16%	OS 5 yr: 96%	⁹⁶
71 9 yr (1.6–27)	Thalassemia: 96% SCA: 4%	MFD: 100%	Bu/Cy/TT Bu IV dosing: EMA nomogram ⁱⁱ + TDM Cy 90–200 mg/kg: 100% TT 10 mg/kg: 38% ST: SCA or MFD	Primary engraftment: 97% ANC(0.5): 20d Platelet(20): 24d Chimerism: 3 mnt: 89% full donor chim 6 mnt: 94% full donor chim Graft failure: 6%	aGvHD: gr 2–3: 30% gr 3–4: 6% ext cGvHD: 12%	Any gr 3–4: 14% Moderate SOS: 1% Liver: 38% Stomatitis: 17% Diarrhea: 10% Hem. cystitis: 7%	OS 3yr: 91% DFS 3yr: 87%	⁹⁷

61	AML	BM: 35%	Bu /Cy or /Mel or /Flu	ANC(0.5): 17 d	aGvHD:	No exact data	OS 2yr: 66%
7.9 yr	CR2: 97%	MD: 71%	Bu Oral: 26% or IV: 74%	Non-engraftment	gr 2-4: 45%	Death due to	OS 4-6yr: 56%
(0.8-21.6)	CR3+: 2%	MMD: 29%	+Cy: 57%	2%	gr 3-4: 18%	Pulmonary tox:	TRM 2yr: 16%
	Refractory:	PBSC: 20%	+Mel: 31%		lim cGvHD: 13%	5%	EFS 2yr: 63%
	2%	MD: 67%	+Flu: 12%		ext cGvHD: 13%		EFS 4yr: 49%
		MMD: 33%	(no dosages available)	Relapse 2yr: 27%			EFS 6yr: 37%
		CB: 45%		Relapse 4-6yr: 31%			
		MD: 8%					
		MMD: 92%					

^a ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, APML: acute promyelocytic leukemia, NHL: non-hodgkin lymphoma, SAA: severe aplastic anemia, SCA: sickle cell anemia, Thalas M: β -thalassemia major, CI: class, CR: complete remission.

^b MSD: matched sibling donor, (M)MFD: (mis)matched family donor, (M)MUD: (mis)matched unrelated donor, MD: matched donor, MMD: mismatched donor, PBSC: peripheral blood stem cells, (U)BM: (unrelated) bone marrow, (U)CB: (unrelated) cord blood.

^c Bu: busulfan, Flu: fludarabine, ATG: anti-thymocyte globulin, Dex: dexamethasone, CY: cyclophosphamide, TT: thiotepa, Amab: alemtuzumab, ST: serotherapy, Mel: melphalan, Hu: hydroxyurea, Aza: azathioprine, IV: intravenous, ibusulfan dosing based on BW described in ⁸⁹, iibusulfan dosing based on EMA nomogram: patient <9 kg: 1.0 mg/kg, 9-16 kg: 1.2 mg/kg, 16-23 kg: 1.1 mg/kg, 23-34 kg: 0.95 mg/kg and >34 kg: 0.8 mg/kg.

^d ANC(0.5): first of 3 consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9/L$, Plts (20) or (50): platelet count $\geq 20 \times 10^9/L$ or $\geq 50 \times 10^9/L$ unsupported for 7 days.

^e aGvHD or cGvHD: acute or chronic GvHD, lim: limited, ext: extensive, gr: grade.

^f Hem. cystitis: hemorrhagic cystitis, tox.: toxicity, iigrowth failure requiring growth hormone replacement.

^g DFS: disease-free survival, EFS: event free survival.

Table 2.2 Overview of recent studies and outcomes in treosulfan-based conditioning before allogeneic HSCT in pediatric patients

Patients (n) and median age (range)	Underlying disease ^a	Donor matching and source ^b	Conditioning regimen ^c	Engraftment and chimerism ^d	GvHD ^e	Toxicities ^f	Survival ^g	Ref
19 3.9 yr (0.3–22)	HLH	MSD: 5% MFD: 26% MUD: 32% MMD: 37% (9/10)	Treo/Flu/TT Treo 42 or 36 g/m ² (<12kg) Flu: 150–180 mg/m ² or 5–6mg/kg TT: 10 mg/kg or 7 mg/kg (<12kg) ST: dose depending on matching	Primary engraftment: 100% Rejection: 11% Chimerism: Full donor: 47% Mixed: 53%	aGvHD: 30% gr 1–2: 23% gr 3: 6%	SOS: 6% (+prophylaxis) Hemorrhage sev: 12% Mucositis gr 3–4: 12% Skin gr 3: 18%	OS: 100% DFS: 100%	⁹⁸
70 0.7 yr (0.1–14.6)	PID	MSD: 11% MFD: 19% UD (7–10/10): 64% HaplID: 6% BM: 57% PBSC: 19% CB: 24%	Treo/Cy or Flu Treo: 36–42 g/m ² Cy 200 mg/kg; 43% Fu 150 mg/m ² ; 57% ST: All except MSD, URD-CB 9/10 (4/6), MSD (3/5), MFD, and 2 nd Tx	Rejection: 3% Mixed, boost: 4% Chimerism 1 yr: Full donor: 57% Stable mixed: 43%	aGvHD: 26% gr 3–4: 10% lim cGvHD: 6%	Seizures: 6% Sev SOS: 3% (Cy) Virus infection: 26% Skin: common Mucositis: mild (no numbers)	OS: 81% TreoFlu: 85% TreoCy: 77%	¹⁴
51 8 yr (0.7–17)	HR or advanced hematologic malignancy: AML, ALL, MDS, NHL, CML, LCH	MSD: 47% UD (9–10/10): 53% BM: 59% PBSC: 37% CB: 4%	Treo/variou Treo: 30–42 g/m ² Cy±VP-16/Mel: 59% Flu±Mel: 35% Mel: 6% Flu: 150–180 mg/m ² Cy: 120 mg/kg Mel: 140 mg/m ² VP-16: 30–40 mg/kg ST: UD recipients	Primary engraftment: 94% ANC(0.5): 17 d Plts(20): 20 d Chimerism: Full donor: 90%	aGvHD: gr 3–4: 18% lim cGvHD: 9% ext cGvHD: 9%	Mucositis: 35% GI: 14% Hepatic: 18% Pulmonary: 2%	NRM: 10% DFS: 67% Relapse: 22% MUD: 8% MSD: 39%	¹²

6	10 mnt (9–11)	ALL infants CR1: 83% CR2: 17%	UCB: 83% 4/6: 17% 5/6: 33% 6/6: 50% MSD, BM: 17%	Treo/Cy Treo: 36–42 g/m ² CY: 60 mg/kg/d 2d ST: 67%	Primary engraftment: 100% ANC(0.5): 18 d Chimerism: Full donor: 83% Mixed >80% donor: 17%	aGvHD≥2: 33%	SOS mild: 17% Mucositis sev: 17% Infections: 67% Microangiopa- thy: 17%	OS: 83% Relapse: 17%	99
50	11 yr (2–21)	Thal M Cl 3 +	MRD: 97% BM: 26% PBSC: 74%	Treo/Flu/TT Treo: 42 g/m ² Flu: 30 mg/m ² /d 4d TT: 8 mg/kg ST: none	Primary engraftment: 94% ANC(0.5): 16 d Plts(20): 21 d Rejection: 8%	aGvHD: 35% gr 2–4: 26% cGvHD: 11%	SOS: 22% (other toxicities not reported)	OS (3yrs): 87% TRM: 12%	16
28	9.6 yr (2–18)	Thal M Cl 1–3	BM: 75% BM+CB: 18% PBSC: 7% Blood group: mismatch: major: 11% bidirectional: 7% minor: 14%	Treo/Flu/TT Treo: 42 g/m ² Flu: 30 mg/m ² /d 4d TT: 8 mg/kg ST: None	ANC(0.5): 15 d Plts(20): 21 d Chimerism: Full donor: 64% Mixed: 18% Rejection: 7%	aGvHD: gr 2–4: 14% lim cGvHD: 10%	Mucositis: gr 1–3: 68% gr 3: 7% SOS sev: 11%	OS (±1yr): 79% TRM: 21%	17

Table 2.2 continues on next page

Table 2.2 *Continued*

Patients (n) and median age (range)	Underlying disease ^a	Donor matching and source ^b	Conditioning regimen ^c	Engraftment and chimerism ^d	GvHD ^e	Toxicities ^f	Survival ^g	Ref
109 8 yr (0.1–20.1)	Malignant and non-malign. disease and also solid tumors	MSD: 22% MFD: 45% MMFD: 15% MUD: 56% MMUD: 2% BM: 49% PBSC: 45% CB: 2% CB+PBSC: 2%	Treo + various Treo: 21–42 g/m ² Flu: 30 mg/m ² /d 5d or 6d 67% TT: 40% Mel: 295% Radiotherapy: 1% ST: 77%	Primary engraftment: 100% WBC (1.0): 20 d Plts(20): 17.5 d Chimerism: Mixed chimera + boost: 3% Rejection: 3%	aGvHD: gr 2–4: 26%	SOS: 3% Skin gr 4: 4% Pulmonary gr 4: 9% Neuro: 9% Seizures: 4% Cardiac: 6.4% Mucositis: mild GI: mild Nappy rash/skin: common	OS (2.5yr): Non-mailgn: 88% Malignant: 49% TRM: 12%	³⁷

^a ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, DBA: Diamond-blackfan anemia, HLH: hemophagocytic lymphohistiocytosis, JMML: juvenile myelomonocytic leukemia, MDS: myelodysplastic syndrome, Non-malign.: non-malignant, PID: primary immune deficiency, Thalas M: β -thalassemia major, HR: high risk, CR: complete remission, CI: class.

^b MSD: matched sibling donor, (M)MFD: (mis)matched family donor, UD: unrelated donor, (M)MUD: (mis)matched unrelated donor, HaploID: haploidentical donor, PBSC: peripheral blood stem cells, BM: bone marrow, CB: cord blood.

^c TREO: treosulfan, CY: Cyclophosphamide, VP-16: etoposide, Flurfludarabine, Melmelpalhan, TT: thiotepa, ST: serotherapy (anti-thymocyte globulin or alemtuzumab).

^d ANC(0.5) or ANC (1.0): first of 3 consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9/L$ or $1.0 \times 10^9/L$, WBC(1.0): White blood cell count of $1000/\mu L$, Plts (20) (50): platelet count $\geq 20 \times 10^9/L$ or $\geq 50 \times 10^9/L$ unsupported for 7 days.

^e aGvHD or cGvHD: acute or chronic GvHD, ext: extensive, lim: limited.

^f GI: gastrointestinal tract toxicity, Neuro: neurological toxicity, gr: grade, sev: severe,

^g RRM: regimen-related mortality, NRM: non-relapse mortality, DFS: disease-free survival.

of the conditioning, RIC was developed to minimize the toxic effects before HSCT. A busulfan-based conditioning with an intermediate dose of 8 mg/kg in total was applied in RIC and treosulfan was introduced as an alkylating agent.¹² In non-malignant disease, busulfan and treosulfan are applied in the same group of patients, in line with current Working Party Inborn Errors/EBMT guidelines. Most experience with treosulfan-based conditioning is gained in primary immune deficiencies (PID) and β -thalassemia. In a large study of 70 pediatric patients with a PID, treosulfan was combined with fludarabine or cyclophosphamide. In these children, a generally mild toxicity profile was observed combined with an overall survival (OS) of more than 80%.¹⁴ The largest study of treosulfan in pediatric patients and young adults with β -thalassemia demonstrated a 5-year OS of 93%, a β -thalassemia-free survival of 84% and no cases of hepatic sinusoidal obstruction syndrome (SOS).¹⁵ The authors suggest that treosulfan-based conditioning is as effective as busulfan-cyclophosphamide-based conditioning and is probably accompanied with less toxicity. However, the long-term effects of treosulfan-based conditioning are not known yet.

Again, there is no study available directly comparing treosulfan- and busulfan-based conditioning in pediatric patients with non-malignant disease. Two studies compared treosulfan, combined with fludarabine and thiotepa, with a retrospective cohort of busulfan (Bu/Cy) in patients with high-risk β -thalassemia.^{16,17} In the first study, TRM and OS were more beneficial in the treosulfan-treated group; OS was 87% in the treosulfan group versus 64% in the busulfan group. The main toxicity was SOS, and the incidence was significantly higher in the Bu/Cy group (66%) compared with the treosulfan-treated group (22%). However, the second study found a higher TRM in the treosulfan group (21%), compared with the busulfan-treated group (0%) and 11% of the treosulfan-treated patients died because of severe SOS. Remarkably, in both studies, especially in the treosulfan group, the incidence of SOS was unusually high.

Therefore, treosulfan is a potentially effective agent with a relatively mild toxicity profile. However, there is a need for a prospective study directly comparing busulfan- and treosulfan-based conditioning in non-malignant diseases. Furthermore, data on the long-term effects of treosulfan in pediatric patients are warranted.

Toxicity of busulfan

Early toxicity

The main early toxicities of busulfan are liver toxicity, pulmonary toxicity, hemorrhagic cystitis, seizures, skin toxicity, diarrhea and mucositis.^{18,19} Busulfan-based regimens are

known to cause liver toxicity, ranging from elevated liver enzymes to SOS. Together with GvHD and infections, SOS is one of the most common early complications after HSCT, occurring in 5–40% of pediatric patients with potentially fatal outcomes.^{20,21} The syndrome is characterized by hepatomegaly, elevated serum bilirubin levels and fluid retention resulting in weight gain. The variability of incidence of SOS can be influenced by the conditioning regimen, patient characteristics, age, underlying disease and existing liver damage.²² High busulfan exposure and busulfan combined with cyclophosphamide is related to an increased risk of SOS.^{23–25} Sufficient time between busulfan and cyclophosphamide administration to allow recovery of glutathione (GSH) depletion or replacement of cyclophosphamide by fludarabine could reduce SOS incidence.²⁶ Furthermore, orally administered busulfan is associated with a higher rates of SOS, because of high variability in exposure and possibly the first-pass effect after oral administration leading to high busulfan concentrations in the small hepatic venules, resulting in damage.²⁷

The incidence of seizures in pediatric patients receiving busulfan has been reported to be between 2% and 10%.^{28,29} It is common practice to administer seizure prophylaxes during busulfan-based conditioning and a variety of antiepileptic drugs has been applied with success in clinical practice.

Late toxicity

Many pediatric allo-HSCT recipients develop long-term complications. Given their improved life expectancy as a result of HSCT, long-term effects are of major concern. In contrast to total-body irradiation, busulfan itself does not cause growth retardation. Growth retardation after busulfan-based conditioning is probably caused by factors such as prior cranial irradiation, underlying disease or long-term use of high doses of glucocorticoids for chronic GvHD.^{30,31} Gonadal dysfunction is a prominent adverse effect after busulfan-based conditioning, especially in young girls, where up to 70% of patients have ovarian failure following busulfan-based HSCT.^{32,33} Furthermore, both hypothyroidism and hyperparathyroidism frequently occur after Bu/Cy-based conditioning for HSCT.³³ Busulfan causes permanent alopecia in up to 50% of the patients and is related to busulfan exposure.³⁴

Toxicity of treosulfan

The main toxicities of treosulfan are mucositis, skin toxicity, diarrhea and hepatic toxicity.^{12,14,35} Mucositis and hepatic toxicity are generally mild compared with busulfan-based conditioning.³⁶ Only rarely does hepatic toxicity develop into SOS and occurrence of SOS is dependent on

pre-HSCT comorbidities and the combination of treosulfan with other alkylating agents (e.g. cyclophosphamide or melphalan).^{12,14} In a recent report, 513 children received treosulfan-based conditioning for their allo-HSCT. The overall SOS incidence was 5%. However, the incidence was higher in patients younger than 6 months (12%).³⁵ In other reports, nappy rash and skin toxicities were reported regularly in infants.³⁷ It is suggested that nappy rashes are probably caused by secondary excretion of the active metabolite l-epoxybutane in the urine.³⁸ Slatter *et al.*¹⁴ reported seizures in treosulfan treated infants after cessation of treosulfan; however, other studies did not report any seizures in such children when patients had pre-HSCT central nervous system (CNS) injury. In general, the use of anticonvulsant prophylaxes before treosulfan is not recommended. However, prophylaxes might be useful in infants.

Given that treosulfan treatment has only recently been introduced for HSCT, only limited data on the long-term effects of treosulfan treatment are available. To date, no long-term toxicities have been reported. Therefore, common long-term toxicities in busulfan-treated patients, such as gonadotoxicity, are to be evaluated in pediatric patients undergoing treosulfan-based conditioning.

PK OF BUSULFAN

Busulfan PK is best described as a one-compartment model. When busulfan is administered orally, absorption is a determinant of high inter- and inpatient variability in exposure. C_{max} generally occurs within 1.5–2.5 h and bioavailability is approximately 70–90%, but highly variable.³⁹ Different factors can cause reduced and variable bioavailability in pediatric patients; the administration of numerous tablets can cause nausea and vomiting and different methods of administration are applied. Crushed tablets are suspended in water, mixed with food or encapsulated to promote swallowing, and tablets can be administered directly or through a gastric tube. Furthermore, a higher gastric pH, differences in transit time and, potentially, a higher first-pass clearance in children can affect bioavailability.⁴⁰ A higher intestinal clearance in children is probably caused by upregulation of glutathione-S-transferase (GST) activity in younger children (0–4 years) compared with older children.⁴¹ Inpatient variability in exposure was reduced after introduction of the intravenous formulation and is generally <15%.⁴² Interpatient variability with an oral formulation can be up to 50% and, when using the intravenous formulation, approximately 20–30% interpatient variability is observed.^{42,43}

Busulfan clearance is age dependent; clearance in pediatric patients is enhanced compared with adults and can be two to four times higher.⁴⁰ In Table 2.3, different PK models are described and an overview of key PK parameters of busulfan in the pediatric population is

Table 2.3 Overview of population pharmacokinetic models recently developed in pediatric patients

Author	Model	Age range (y)	Clearance (Cl)	Volume of distribution(V)	Dosing accuracy and IIV/IOV	Ref
Trame	One-compartment model with 1 st order absorption	0.4–18.8	4.16 L/h/m ² 4.11 L/h/kg ^{0.75}	15.2 L/kg	Dose = 19.6 × BSA Dose = 19.4 × (BW/27.2) ^{0.75} 75–80% of patients within target AUC (900–1500 µM*min)	51
	2 models 1. Cl: corrected for BSA 2. Cl allometric scaled for BW Scaling exponent: 0.75 V in both models as function of BW					
Bartelink	Two-compartment model Cl and V allometric scaled BW Variable scaling exponent Cl, based on BW: L1*BW ^m = 1.2 - 0.55 $Cl = Cl_{15.3kg} * (BW/15.3)^{L1 * BW^{-N}}$ Scaling exponent V = 0.89(fixed)	0.1–26	3.47 L/h L1 = 1.56 M = -0.226	11.1 L	Model based nomogram: Clearance: IOV 15% IIV 27%	89
	One-compartment model Cl and V allometric scaled for BW Scaling exponent for Cl: < 9 kg = 1.25 ≥ 9 kg = 0.76 Scaling exponent for V: 0.86					
Paci	One-compartment model Cl and V allometric scaled for BW Scaling exponent for Cl: < 9 kg = 1.25 ≥ 9 kg = 0.76 Scaling exponent for V: 0.86	0.03–15	2.18 L/h	nd	IIV Cl 23% V 22% IOV Cl 11%	100
Savic	One-compartment model Cl and V allometric scaled for BW Scaling exponent Vd = 1.0 Cl = 0.75 Cl also corrected for maturation effect: $Cl_i = Cl_{pop} \left(m + (1 - m) * \left[1 - e^{-age * K_{mat}} \right] \right) * (BW / 8 kg)^{0.75}$ m = maturation magnitude effect for age K _{mat} = maturation rate constant	0.08–3.3	2.3 L/h m = 0.46 K _{mat} = 1.4	6.4 L/kg	IIV Cl 25% V 25%	53

BSA: body surface area (m²), IIV: interindividual variability, IOV: interoccasion variability, Allometric scaling based on body weight occurs according to the following formula: BW_i^x / BW_{pop} in which BW_i is the body weight of the individual, BW_{pop} is the body weight of a typical individual set as such in the model and x the scaling exponent.

provided. Busulfan is metabolized by conjugation with glutathione, resulting in formation of a glutathione conjugate. This reaction is catalyzed by GSTA1, GSTM1 and GSTP1, and occurs in the liver and intestine. GSTA1 is the predominant GST enzyme involved in busulfan metabolism; GSTM1 and GSTP1 have 46% and 18% of the activity of GSTA1 in busulfan metabolism, respectively.⁴⁴ The glutathione-conjugate dissociates into γ -glutamyldehydroalanylglycine and tetrahydrothiophene (THT). THT is oxidized into sulfolane and subsequently into 3-hydroxy sulfolane (Figure 2.2).^{45,46} It is suggested that cytochrome P450 enzymes are involved in the oxidation of THT and sulfolane.⁴⁷ Furthermore, it is suggested that transporters are involved in active transport of the glutathione conjugate out of the cell (Figure 2.2).^{48,49}

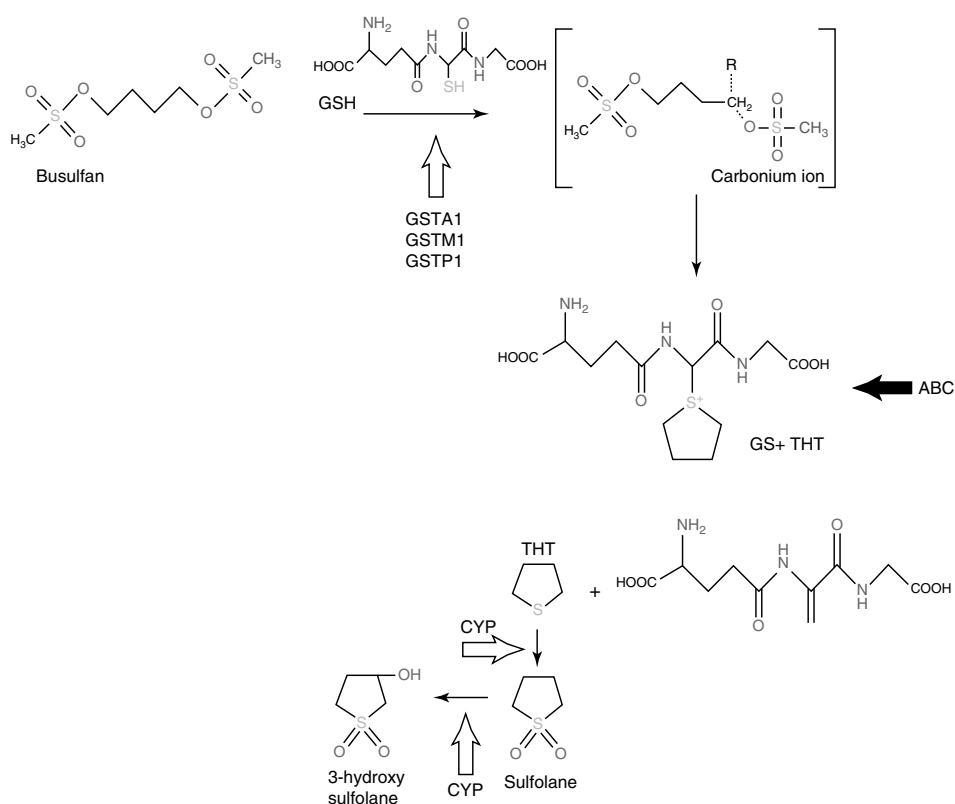


Figure 2.2 Metabolism of busulfan and potential involvement of enzymes and transporters. Busulfan conjugation with glutathione (GSH) is catalyzed by glutathione-S-transferases (GST). The conjugate dissociates into γ -glutamyldehydroalanylglycine and tetrahydrothiophene (THT). THT is oxidized into sulfolane and subsequently into 3-hydroxy sulfolane. GSTs are involved in the conjugation of busulfan with GSH. It is suggested cytochrome P450 enzymes (CYPs) are involved in the oxidation of THT and sulfolane and transporters are involved in active transport of the glutathione conjugate out of the cell. ABC: ATP-binding cassette transporters.

Factors influencing busulfan PK

Children have an increased busulfan clearance compared with adults, which is partially caused by an increased liver size to body weight (BW) ratio.⁵⁰ Therefore, clearance should be expressed by body surface area or allometrically scaled BW to account for this age-related variability in hepatic function.^{47,51} These measures do not account for all the differences in busulfan clearance between adults and pediatric patients. It is demonstrated that younger children (2–4 year) have an elevated ratio THT+ (the metabolite of busulfan) to busulfan, because of enhanced conjugation of busulfan compared with older children and adults. No elevated GSH levels were measured and it was suggested that children have an increased metabolism due to higher expression or activity of GST rather than through more available GSH.⁵² These processes have been incorporated in population PK models (Table 2.3). Furthermore, Savic *et al.*⁵³ developed a population PK model for busulfan in infants (<12 kg). In the model, BW was allometrically scaled as a measure of physiological growth. Age was also incorporated into the model to account for maturation of enzyme function during the first 2 years of life. In infants, clearance increased 1.7-fold between 6 weeks and 2 years of life. Overall, maturation in the GST expression or activity during the first 2 years of age is likely to have an important role and GST activity reaches a maximum by the age of 2 years.

Several investigators studied the effect of polymorphisms in the genes encoding GSTs involved in busulfan metabolism. The first study in pediatric patients demonstrated that children who were heterozygous or homozygous for the *GSTA1**B haplotype (regardless of age) exhibited a 30% decrease in busulfan clearance after intravenous administration.⁵⁴ However, other groups did not find an association of *GSTA1* genotype with busulfan clearance, or only in patients receiving busulfan orally.^{23,55,56} There are also conflicting results on the effect of the *GSTM1* genotype in relation to busulfan PK; one study demonstrated a lower busulfan clearance in patients with the *GSTM1*-null genotype,²³ which is conflicting with the findings of Srivastava *et al.*⁵⁷, who demonstrated a higher clearance and risk of SOS in *GSTM1*-null patients. The authors suggest that toxicity is caused by the metabolite rather than by busulfan itself. Several factors could account for these conflicting results, including the route of administration, the age range of patients and the way clearance is expressed. If clearance is not adjusted for body size, the interpatient variability in clearance is larger and the effect of body size dominates the probably smaller effect of the genetic variants.

Apart from GSTs, the involvement of other enzymes and transporters has been suggested.^{48,49} Polymorphisms in transporters and other enzymes in relation to busulfan PK have been studied; Krivoy *et al.*⁵⁸ found a combined association of the *GSTM1* and ATP-binding cassette, sub-family B

(MDR/TAP), member 1 (*ABCB1*) genotype and oral busulfan clearance in adults and another study demonstrated the involvement of *CYP2C9* and *CYP2B6* in busulfan metabolism.⁴⁸ Furthermore, in a recent study, 40 genetic polymorphisms, including several genes encoding drug transporters and CYP enzymes, in relation to HSCT outcome were studied.⁵⁹ Only one polymorphism in *GSTA2* was related to OS and TRM. In most of the PG studies on busulfan PK, a small set of polymorphisms was investigated that were likely to be involved in busulfan metabolism, according to the candidate gene approach. To further investigate the PG contribution to interpatient variability in busulfan PK, a shift from the candidate gene approach to a more broad PG analysis should be made.

In several studies, clearances between different disease groups were compared. It is suggested that patients with β -thalassemia have an increased and highly variable busulfan clearance caused by an iron overload inducing GST activation and pre-HSCT liver injury.⁶⁰ Pediatric patients with inherited diseases were associated with a low and highly variable busulfan clearance.⁶¹ This could have been caused by the young age of the patients (mean age < 1 year) and co-medication. Hence, the role of underlying disease on busulfan PK is not clear, given that different studies demonstrate conflicting results.

Of the regularly administered co-medication, theoretically phenytoin, itraconazole, acetaminophen and metronidazole are associated with altered busulfan clearance.^{62–64} Itraconazole is a strong inhibitor and phenytoin a strong inducer of cytochrome P450 enzymes, but its effect on busulfan metabolism is not clear. Phenytoin is often replaced with a different anticonvulsant, such as levetiracetam or clonazepam. The effect of itraconazole on busulfan exposure in clinical practice is not always evident; in one study, no influence of itraconazole on busulfan exposure was observed.⁶⁵ The effect of metronidazole on busulfan PK is probably caused by GSH depletion by the metronidazole reactive metabolites.⁶⁴ However, clinical evidence is missing, and metronidazole is rarely combined with busulfan. Hence, several interacting drugs for busulfan have been identified, although their effects in clinical practice are limited.

Analytical methods

To relate a PK profile of a drug to outcome and to guide dosing based on exposure, it is essential to have a proper bioanalytical method. Any method applied should be accurate and precise over the whole concentration range; the lower limit of quantification (LOQ) and concentration range should be adequate for busulfan determination in clinical practice and the method should be selective. Furthermore, relative short run times and sample preparations are important, because of the short course of busulfan. An overview of bioanalytical methods for determination of busulfan, cited in recent literature on busulfan, is given in Table 2.4. When

Table 2.4 Overview of bioanalytical methods for determination of busulfan, cited in recent literature on busulfan

Author	Method	Concentration range	Samples quantity	Run time	Derivatization time	Estimated total time	Ref
Kellog	LC-MSMS	123–2463 µg/L	50 µL plasma	3 min	NA	2 hour for 10 samples	¹⁰¹
Mürdter	LS-MS	10–2000 µg/L	200 µL plasma	10 min	NA	Unknown	⁸⁸
Quernin	GC-MS	20–2000 µg/L	1000 µL plasma	14 min	2 hour	Unknown	¹⁰²
Lai	GC-MS	40–4000 µL/L	1000 µL blood	12 min	1 hour	24h for 40 samples 3 h manual labour	⁶⁶
Bleyzac	HPLC-UV	100–2000 µg/L	200 µL plasma	10 min	Direct	32 minutes per samples	¹⁰³
Cremers	HPLC-UV	30–8000 µg/L	200 µL serum	10 min	Direct	2 hour for 10 samples	⁶⁷

busulfan is administered daily, it is completely eliminated and, therefore, the LOQ of a method should be as low as possible, but at least 40 µg/L. In HPLC-UV and GC-MS analytical assays, derivatization of busulfan is conducted to make the compound detectable. This procedure is selective; only derivatized compounds are detected.^{66,67} However, it can be a time consuming step; for example, in GC-MS, derivatization steps can take up to 2 hours. Another essential part is the required volume of patient material (plasma or serum), especially in young children. A limited sampling model is a helpful method to reduce the required total blood volume and, with that, patient burden. In modern LC-MSMS methods, 50–100 µL of serum or plasma can be sufficient. Older methods require up to 1000 µL. Overall, LC–MSMS methods are the preferred methods because of the smaller volumes required, shorter run times and simple sample preparation. However, disadvantages of these methods include the complexity and high costs of equipment.

PK and clinical outcome

For oral busulfan, several investigators demonstrated a relation between busulfan exposure and clinical outcome. In adults, high exposure was related to toxicity, especially SOS, and lower exposure was related to rejection of the transplant.⁶⁸

However, in children, this relation was not that evident, which is likely to be the result of a lower overall exposure in pediatric patients because of an enhanced clearance, resulting in less toxicity and lower SOS incidence. Furthermore, soon after the relation between exposure and clinical outcome was established, therapeutic drug monitoring (TDM) was introduced to

optimize exposure, thereby reducing interpatient variability.⁶⁹ However, there are several studies demonstrating an association between exposure and clinical outcome in pediatric patients. Ljungman *et al.*⁷⁰ investigated the busulfan concentration at steady state (C_{ss}) in a cohort of adults and pediatric patients and demonstrated that a $C_{ss} > 721$ ng/mL was associated with increased TRM and OS. Furthermore, in two pediatric cohorts, $C_{ss} > 600$ ng/mL was associated with a higher probability of engraftment.^{71,72} Unfortunately, these studies provided no target for busulfan exposure. Finding the optimal target for busulfan is hampered by the application of different measures of exposure: C_{ss} in ng/mL, area under curve (AUC) in $\mu\text{M} \cdot \text{min}$ or $\text{mg} \cdot \text{h/L}$. Furthermore, these measures can be expressed per dose or cumulative over a 4-day course. In addition, busulfan can be administered one, two or four times daily, which results in different exposure levels per dose. Therefore, we advocate that busulfan exposure is expressed as a cumulative exposure over a 4-day course to make exposures more easily comparable.

Bartelink *et al.*⁷³ attempted to find the optimal busulfan AUC in pediatric patients receiving Bu/Cy-based conditioning. Exposure proved related to event free survival (EFS), graft failure and relapse, and the authors advocated targeting busulfan to a narrow therapeutic range of 74–82 $\text{mg} \cdot \text{h/L}$. Many studies applied an AUC target of 900–1350 $\mu\text{M} \cdot \text{min}/\text{dose}$, which is equivalent to a total AUC of 57.6–86.4 mg h/L which is a broad target compared with that defined by Bartelink *et al.*⁷³

Using TDM, the total exposure can be optimally guided toward a narrow range. In a recent study, the cumulative exposure over a four day course was not related to clinical outcome and 90% of the patients were within the target range.⁷⁴ However, in this study, a C_{ss} after the first dose below 600 ng/mL was associated with increased OS and EFS and decreased non-relapse mortality. According to the authors, a higher first dose C_{ss} causes increased depletion of GSH, resulting in decreased metabolism of cyclophosphamide and increased toxicity.

Overall, busulfan exposure is related to clinical outcome and tight control of exposure could improve HSCT outcome after busulfan-based conditioning. Furthermore, the target exposure of busulfan depends on the regimen it is applied in. Optimal targets have mainly been investigated in Bu/Cy regimens. The optimal range for busulfan exposure in more recent applied combinations, such as busulfan and fludarabine, is more difficult to define, because of the upfront targeting of busulfan. In a recent study on busulfan–fludarabine-based conditioning in pediatric patients, the target AUC for busulfan was 80–95 mg h/L . This conditioning regimen proved to have a favorable toxicity profile compared with Bu/Cy (Mel) conditioning and was equally effective.⁷⁵ Moreover, more individualized target exposures should be defined, depending on the total conditioning regimen and the disease type of the patient.

PK OF TREOSULFAN

Treosulfan is non-enzymatically converted into its active metabolites; a mono- and diepoxide.⁷ The two metabolites are excreted in the urine and no enzyme or transporter is known to be involved in treosulfan metabolism. There are several studies investigating the PK of treosulfan in patients, most of which were performed in adults. In almost all the studies, a linear relation between dose and exposure of treosulfan was found. Yet, the exposures demonstrated in the different studies are not in accordance with each other. The PK of treosulfan was first analyzed in adult patients with solid tumors.^{76,77} Patients received 8 or 10 g/m², which is a typical dose in solid tumors,⁷⁶ or 20–56 g/m² with stem cell support.⁷⁷ The half-life of treosulfan was 1.8 hour⁷⁶ and 2.0 hour⁷⁷ and urinary excretion of the parent compound was approximately 25%. Half-life and AUC were somewhat increased in patients receiving the highest dose of 56 g/m², possibly because of acidotic changes in plasma leading to the decreased formation of active metabolites. Both studies demonstrated a linear relation between dose and exposure. After 10 g/m², a mean AUC_{0–24h} of 977 ± 182 µg/ml*h was observed and a dose of 20 g/m² resulted in a mean AUC of 2325 µg/ml*h. In a third study in adult patients receiving 12 or 14 g/m² of treosulfan before SCT, a mean treosulfan exposure of 898 ± 104 µg/ml*h and 1104 ± 173 µg/ml*h, respectively, was observed.⁷⁸ Renal excretion of treosulfan was approximately 39%, a half-life that was comparable to earlier studies. However, exposures after 12 and 14 g/m² were comparable to the earlier observed AUC after a dose of 10 g/m². In another study with HSCT recipients, pediatric patients were also included. The mean AUC levels were higher than in the studies with solely adult patients, although the AUC levels after two different doses were similar: 1365 ± 293 µg/ml*h and 1309 ± 262 µg/ml*h, for 12 g/m² and 14 g/m², respectively.⁷⁹ Therefore, the authors suggested that increased doses would be unlikely to lead to increased efficacy.

PK studies of treosulfan in pediatric populations are limited. Glowka *et al.*⁸⁰ described the PK of a set of seven pediatric patients. In this study, three different dosages were administered: 10 g/m² (n = 1), 12 g/m² (n = 5) and 14 g/m² (n = 1) daily. Treosulfan PK was best described by a two-compartment disposition model with first-order elimination. Interpatient variability in patients receiving 12 g/m² was large, with a coefficient of variation of 70%. The exposure in the single patient receiving 14 g/m² was relatively high (1960 µg/ml*h) when considering a linear relation between dose and exposure. This high AUC could result from metabolic acidosis as a result of methanesulfonic acid formation resulting in a reduction of treosulfan conversion into the active derivatives.

There are some conflicting results between the different PK studies in adults and pediatric patients and, therefore, larger pediatric cohorts should be investigated. At this point, dose optimization-based PK of treosulfan is of interest, especially in pediatric patients.

Analytical methods

The PK data regarding treosulfan are scarce, which could be because of the limited availability of an analytical method for determination of treosulfan. Although treosulfan is a prodrug, most bioanalytical methods are aimed at analyzing treosulfan itself. Treosulfan is non-enzymatically converted into its active metabolites. Therefore, it is suggested that the concentration of treosulfan itself is a good representation of the alkylating activity.⁷⁶ Most analytical methods are based on reversed phase-HPLC methods (rp-HPLC) with refractometric detection.^{77,81} To make treosulfan (and its metabolites) detectable via UV detection, treosulfan can be derivatized.⁸² UV-detection is a more selective and sensitive detection method, although derivatization can be time consuming. The conversion of treosulfan is pH dependent; therefore, in most studies, blood samples were acidified directly after collection by adding citric acid. This could be considered a complicated logistics step that might hamper sample collection in clinical practice.

PK and clinical outcome

Data of treosulfan exposure in relation to clinical outcome are scarce. Moreover, most of the studies on treosulfan PK have only limited patient numbers and insufficient power to assess an association with clinical outcome, although attempts have been made to relate clinical outcome to treosulfan dose. Scheulen *et al.*⁷⁷ performed a dose escalation study to assess the maximum tolerated dose. Doses higher than 47 g/m² were associated with severe toxicities. Normally in treosulfan-based conditioning, treosulfan doses up to 42 g/m² are divided over 3 days. It is unknown whether toxicities are comparable when the cumulative dose is administered as one dose or when it is divided over multiple days.

In a small adult patient population (n = 18) with malignant disease, the PK and clinical outcome of two different doses of treosulfan (36 and 42 g/m², cumulative dose) were studied. There were no differences in clinical outcome (engraftment, non-relapse mortality and OS) between the two doses.⁷⁸ Different doses of treosulfan in pediatric patients undergoing HSCT because of malignant diseases (myeloid and lymphoid, n = 51) have been studied.¹² In this study, the higher dose of treosulfan (total 42 g/m²) was more beneficial in relation to donor engraftment and hematopoietic chimerism. Nevertheless, there was no significant difference in DFS in patients receiving different doses of treosulfan.

The cumulative dose of 42 g/m² is a common dose regimen for treosulfan-based conditioning; toxicity is mild and engraftment might be enhanced at this dosing level. However, the outcome of HSCT is based on many factors and the most appropriate dose remains to be elucidated.

Whether exposure is a better marker for treosulfan efficacy and safety than the cumulative dose, as observed in busulfan, needs to be further investigated. Furthermore, in clinical practice, children under 1 year of age frequently receive a reduced dose of 30–36 g/m². However, there is currently no evidence supporting this practice.

DOSE OPTIMIZATION OF BUSULFAN

Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is of clinical value for drugs with a narrow therapeutic index, a clear relation between exposure and clinical outcome, substantial interpatient variability and small inpatient variability, repeated administration, absence of alternative laboratory parameter and an appropriate analytical method.⁸³

Busulfan meets all the criteria for applying TDM, except for inpatient variability in oral busulfan, which is relatively large. Lindley *et al.* applied a test dose to predict busulfan exposure after oral administration and, in only 46% of the patients, the apparent oral clearance predicted the appropriate dose to achieve the target AUC.⁸⁴ After intravenous administration, the inpatient variability was significantly reduced and, in a higher percentage of the patients, the target exposure was reached when TDM was applied.⁸⁵ Therefore, TDM-guided dosing is more useful in an intravenous regimen of busulfan. Furthermore, because of the short treatment course with a maximum of 4 days, it is necessary to have sufficient expertise and a proper logistic process in place for TDM-guided dosing of busulfan.

Especially in pediatric patients, it is essential to limit the burden of TDM caused by serial blood sampling. Therefore, the application of a limited sampling model can help to achieve this goal.

Based on two to four plasma-level measurements, the exposure to busulfan can be adequately estimated. Many published limited sampling models are based on a regression algorithm. Regression algorithms are capable of predicting the busulfan exposure accurately and precisely using concentrations measurements in three samples.⁸⁶ However, the disadvantage of these algorithms is the necessity of sampling at the exact time points included in the algorithm. Small deviations in the sampling time point can result in significant deviations in predicted AUC. In addition, Bayesian PK procedures have been used to determine busulfan exposure. These procedures provide more flexibility in sampling times and, with that, higher accuracy in exposure estimation.⁶⁷

Dosing nomograms and population PK modelling

A population PK model (pop-PK model) can be constructed to describe the PK profile of a drug in the population, incorporating patient characteristics that contribute to the interpatient variability. From these PK models, dosing nomograms can be extracted, ultimately leading to target exposure in individual patients without requiring TDM or at least rapid achievement of target AUC. Nguyen *et al.* developed a five-step nomogram for intravenous busulfan with different doses based on body weight of the patient (<9 kg, 1.0 mg/kg; 9–16 kg, 1.2 mg/kg; 16–23 kg, 1.1 mg/kg; 23–34 kg, 0.95 mg/kg; and >34 kg, 0.8 mg/kg).⁸⁷ This nomogram is the official dosing recommendation of the European Medical Agency (EMA), although studies have demonstrated that, with this dosing nomogram, a large portion of the patients do not reach the target AUC and TDM remains necessary to individualize the dose.⁸⁸ Recent models apply allometric scaling of the PK parameters clearance and volume of distribution. For clearance, the scaling component can be either fixed at 0.75 or the exponent can be varied based on the age or weight of the individual. The latter approach results in a higher exponent in infants and neonates, which accounts for a faster increase in clearance with growth and maturation. Not only body size, but also an increase in GST activity causes this phenomenon.^{53,89} Dosing simulations based on the recently developed pop-PK models (Table 2.3) demonstrate that a large proportion of patients theoretically reach the target exposure. However, the proposed regimens have not been validated prospectively in independent cohorts. Furthermore, approximately 25% of the interpatient variability remains unexplained, suggesting that TDM is still a necessity to further optimize busulfan dosing.

DOSE OPTIMIZATION OF TREOSULFAN

In a phase I dose escalation study in adults, it was demonstrated that a dose of 47 g/m² is the maximum tolerated dose of treosulfan.⁷⁷ Furthermore, in a few studies, different doses of treosulfan in patients undergoing HSCT were applied, and relations with clinical outcome were assessed. However, most of these studies were limited by sample size⁷⁸ or highly variable patient characteristics¹² and no definite conclusion about the optimal dose can be made based on them.

It is essential to further study the PK and PD profile of treosulfan in pediatric patients. It seems more rational to choose the treosulfan dose based on the underlying disease and condition of the patient. In malignant diseases and, for example, β -thalassemia, a myeloablative regimen is required to give a maximum reduction of the malignancy and autologous hematopoiesis and to allow adequate donor engraftment. Treosulfan was first applied in allo-HSCT as a reduced intensity regimen. However, aplasia induced by treosulfan is rapid and sustained, and engraftment after high doses of treosulfan is prompt, resulting in early achievement of full donor

chimerism.⁹⁰ To date, it is not clear which dose of treosulfan is more likely to be a RIC or MAC, and which other factors are involved. For example, other drugs in the conditioning regimen and age of the patient and, with that, the level of treosulfan exposure could contribute to the myeloablative potency and toxicity profile of treosulfan.

CONCLUDING REMARKS

Busulfan and treosulfan are both effective in conditioning before HSCT in pediatric patients. The experience with busulfan is broader than with treosulfan, and many studies have demonstrated that TDM-based dosing is pivotal to achieve optimal busulfan exposure and, thus, clinical outcome. Furthermore, improved understanding of maturation processes in children, the development of pop-PK models and PG studies has revealed factors accounting for interpatient variability in busulfan exposure. The role of PG markers in the efficacy of busulfan should be further elucidated by broader PG analyses and in larger cohorts. Additionally, the effect of genetic markers could be incorporated in pop-PK models and could explain a portion of the remaining interpatient variability.

Knowledge of the treosulfan PK profile in pediatric patients is limited and PK-PD studies are necessary to optimize the dose. The first steps to increase this knowledge have been made. It is essential to have a proper bioanalytical method and population PK methods should be developed. At this point, PG analysis on treosulfan metabolism is probably not relevant because no metabolic enzymes or transporters appear to be involved in treosulfan PK.

The two agents were not prospectively compared in children with either malignant or non-malignant disease. Therefore, trials comparing both agents in well-defined patient groups are warranted. The development of a new study in pediatric patients with acute lymphoblastic leukemia in which treosulfan and busulfan are randomized versus total-body irradiation based conditioning is important. In this study, primary outcome will be mainly focused on relapse risk.

In addition, the usefulness of TDM and PG to achieve optimal clinical outcome is mainly based on short-term outcome parameters, such as relapse, engraftment and GvHD, whereas, for both alkylating agents, long-term outcome parameters (such as longterm survival and late toxicities) are also important. In the long run, given the potential long-term toxicity of chemotherapeutic agents, one would like to define the lowest dose level at which these drugs are effective in the various disease situations, thus limiting adverse effects as much as possible. In conclusion, better understanding of the PK and PG of busulfan and treosulfan has the potential to further improve the outcome of pediatric allo-HSCT.

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