Treosulfan pharmacokinetics and dynamics in pediatric allogeneic stem cell transplantation

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CHAPTER 06

EFFECT OF BUSULFAN AND TREOSULFAN ON GONADAL FUNCTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN AND ADOLESCENTS WITH NONMALIGNANT DISEASES IS NOT EXPOSURE-DEPENDENT

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ABSTRACT

With an increasing number of young patients surviving into adulthood after hematopoietic stem cell transplantation (HSCT), gonadal dysfunction becomes an important late effect with significant impact on quality of life. In this retrospective single-center study, we evaluated the exposure of busulfan (BU) and treosulfan (TREO) in relation to gonadal function in pediatric patients transplanted for a nonmalignant disease between 1997 and 2018. In the BU cohort, 56 patients could be evaluated and gonadal dysfunction occurred in 35 (63%) patients. Lower BU exposure (cumulative area under the curve cAUC <70 mg*h/L) was not associated with a reduced risk of gonadal dysfunction (OR 0.92 95% confidence interval (CI) 0.25-3.49, p=0.90). In the TREO cohort, 32 patients were evaluable and gonadal insufficiency occurred in 9 patients (28%). Lower TREO exposure (AUC <1750 mg*h/L on day 1) was not associated with a reduced risk of gonadal dysfunction (OR 1.6 95%CI 0.16-36.6, p=0.71). Our data do not support the premise that reduced intensity BU-based conditioning lowers the risk for gonadal toxicity and it is unlikely that TDM-based reduced treosulfan exposure will further reduce the risk for gonadal dysfunction.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment option for a growing number of nonmalignant indications in childhood. Increased safety and effectiveness of the transplant procedures and particularly conditioning regimens have contributed to this rise in transplants in the last decade [1]. The focus, when improving the conditioning regimen, has mainly been on decreasing acute toxicity, while trying to maintain efficacy. Using less toxic agents, less toxic combinations, dose optimization and personalized dosing with the help of therapeutic drug monitoring (TDM) or model informed precision dosing (MIPD) have shown to be successful strategies to achieve this goal [2-4]. The late effects of the transplant procedure, such as gonadal dysfunction and growth impairment, become more important as an increasing number of (very) young patients are transplanted, that benefit from the curative potential of HSCT and survive into adulthood [5]. In a recent study, we reported a high prevalence of endocrine complications in survivors of pediatric HSCT in nonmalignant diseases [6]. Female patients were more likely to develop gonadal dysfunction after busulfan-based (BU) conditioning compared to treosulfan-based (TREO) conditioning. To date, it is unknown if drug exposure is of influence on the prevalence of endocrine complications. In this study we retrospectively evaluated if the exposure of busulfan and treosulfan was related to the risk of gonadal dysfunction in pediatric patients transplanted for a nonmalignant disease.

METHODS

Study population and design

This retrospective non-interventional single-center study included patients with a nonmalignant disease who received BU- or TREO-based conditioning prior to HSCT in line with the respective EBMT Working Party and institutional guidelines at the department of Pediatrics at the Leiden University Medical Center in the Netherlands between 1997 and 2018. Exclusion criteria were re-transplantation, no data available on the outcome measures of the study and death within two years post-transplant. The

study protocol was assessed by the local medical ethical committee who determined that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study. The need for informed consent was waived.

Data collection

All patients underwent a clinical and laboratory endocrine evaluation prior to HSCT. At annual follow-up visits after HSCT, pubertal stage was evaluated and laboratory investigations including FSH, LH, testosterone and estradiol, were performed. Patient and transplant characteristics were collected from the medical files including sex, age, underlying disease and conditioning regimen. Plasma serum concentrations of BU and TREO were collected if available. Indications for HSCT were classified as inborn errors of immunity or metabolism (IEI/IEM), hemoglobinopathies (HBP) and bone marrow failure (BMF). Data on gonadal dysfunction were collected up until last follow-up.

Busulfan and treosulfan pharmacokinetics

Validated analytical methods were used to quantify BU and TREO in serum and described earlier [7-9]. TREO area under the curve (AUC) on day 1 as a measure of total exposure was estimated with a pharmacokinetic model using the posthoc estimation function in NONMEM [10]. BU cumulative AUC (cAUC) was estimated using a validated limited sampling model [7]. Empirical Bayesian PK parameter estimates at steady state (clearance and volume of distribution) were generated for all individual children using the PK software package MwPharm, University of Groningen, The Netherlands [11]. The AUC was calculated from the expression dose/clearance (CL).

Outcomes

Gonadal dysfunction was defined as gonadotropins above the reference range, i.e. FSH ≥ 21.5 U/L and/or LH ≥ 60 U/L for females and FSH ≥ 12.5 U/L and/or LH ≥ 9.0 U/L for men. If elevated gonadotropins had normalized at subsequent visits gonadal dysfunction was classified as transient; if they remained elevated at last visit it

was classified as permanent [12]. Patients at Tanner stage ≥G2 or ≥B2 were classified as (post)pubertal and were included in the analysis [13, 14]. Patients diagnosed with gonadal dysfunction before HSCT were excluded from this analysis.

Statistical analysis

Descriptive statistics were performed on the data. Normally distributed continuous parameters are shown as mean ± standard deviation, all log-normally continuous distributed parameters as median and interquartile range (IQR) and categorical variables as frequency (percentage). BU and TREO exposure was divided in 2 exposure groups; low (< 70 mg*h/L for BU and < 1750 mg*h/L for TREO) and high (≥ 70 mg*h/L for BU and ≥ 1750 mg*h/L for TREO). For BU this is based on recommended targets for myeloablative (85-95 mg*h/L) and reduced intensive conditioning (60-70 mg*h/L) [15]. For TREO, this is based on results published earlier on exposure and acute toxicity and clinical outcome [9]. Univariate logistic regression analyses were performed to evaluate BU and TREO exposure as a risk factor for outcome. BU and TREO exposure was tested as discrete variable, considering low and high exposure groups. All *p*-values were 2-tailed and considered significant when $p < .05$. Statistical analyses were performed with R (version 4.1.0) and R studio version 1.4.1717.

RESULTS

Patient characteristics

A total of 157 patients were included, 90 were conditioned with BU and 67 with TREO. Of the 90 patients in the BU cohort, 56 patients were eligible for analysis; 27 patients were still prepubertal and data of 7 patients were incomplete or were excluded from the analysis because of gonadal dysfunction prior to HSCT. Of the 67 patients in the TREO cohort, 32 patients were eligible for analysis; 34 patients were still prepubertal and data of 1 patient was incomplete. Patient and transplant characteristics are shown in Table 1. In the BU group, the majority of patients were conditioned with BU in combination with cyclophosphamide (48%), followed by BU and cyclophosphamide in combination

with another agent (melphalan, etoposide or fludarabine) (23%). In the TREO group, the majority was conditioned with TREO in combination with fludarabine and thiotepa (59%), followed by TREO with fludarabine (25%). Exposure data of 41 (68%) and 19 (59%) patients was available in the BU and TREO group, respectively.

Table 1. Patient and transplant characteristics

	Eligible BU patients (N=56)	**Eligible TREO patients (N=32)**
Characteristic		
Sex (n: M/F)	39/17	16/16
Age (years, median (IQR))	5.6 (3.2-11.3)	13.5 (8.7-15.0)
Age at last follow up (years, median (IQR))	18.2 (15.4-20.6)	16.6 (15.3-18.6)
Length of follow up (years, median (IQR))	11.4 (8.3-17.1)	4.0 (2.5-8.3)
Diagnosis for HSCT		
Inborn errors of immunity (%)	31 (55)	5 (16)
Hemoglobinopathies (%)	15 (27)	24 (75)
Bone marrow failure (%)	10 (18)	3 (9)
Donor		
MSD (%)	22 (39)	15 (47)
MUD (%)	27 (48)	13 (41)
MMFD/Haplo (%)	7 (12)	3 (9)
ORD (%)	0 (0)	1 (3)
Pubertal status at HSCT		
Prepubertal (%)	43 (77)	15 (47)
(Post)pubertal (%)	13 (23)	17 (53)
Combining agents		
Fludarabine (%)	10 (18)	8 (25)
Fludarabine + Thiotepa/Melphalan (%)	6 (11)	19 (59)
Cyclophosphamide (%)	27 (48)	0
Cyclophosphamide + Melphalan/Etoposide/Flu (%)	13 (23)	5 (16)
Exposure measured (%)	41 (68)	19 (59)
Low (<70 mg*h/L for BU, <1750 mg*h/L for TREO) (%)	27 (66)	5 (26)
High (≥70 mg*h/L for BU, ≥1750 mg*h/L for TREO) (%)	14 (34)	14 (74)

MSD: matched sibling donor, MUD: matched unrelated donor, MMFD: mismatched family donor, ORD: other related donor.

Gonadal dysfunction in BU-treated patients

At time of HSCT, 43 (77%) of 56 patients were prepubertal and 13 (23%) were (post) pubertal (Table 2). Median age at HSCT was 5.6 years (IQR 3.2-11.3) and median

age at last visit was 18.2 years (IQR 15.4-20.6). Gonadal dysfunction occurred in 35 (63%) patients, 19 male and 16 female patients. In 4 patients (2 male and 2 female), gonadal dysfunction was transient of whom one female patient needed temporary hormonal substitution. When comparing BU + Cyclophosphamide and BU + Fludarabine, permanent gonadal dysfunction occurred in 50% of evaluable patients in both groups. BU exposure data was available of 41 patients. Lower BU exposure (cAUC < 70 mg*h/L) was not associated with a reduced risk of gonadal dysfunction (OR 0.92 95% confidence interval (CI) 0.25-3.49, p=0.90). The distribution of BU exposure in relation to gonadal function is shown in Figure 1A.

Table 2. Gonadal dysfunction in BU-treated patients

	No (N = 21)	**Yes (N=31)**	**Transient (N=4)**
Characteristic			
Sex (n: M/F)	20/1	17/14	2/2
Age (years, median (IQR))	5.5 (3.2-7.7)	7.4 (3.5-14.0)	2.9 (1.1-6.2)
Age at last follow up (years, median (IQR))	16.6 (13.7-20.6)	19.7 (16.6-23.1)	18.9 (16.8-21.7)
Length of follow up (years, median (IQR))	13.3 (8.9-16.9)	10.9 (7.8-18.4)	15.2 (12.4-18.0)
Diagnosis for HSCT			
Inborn errors of immunity (%)	14 (67)	14 (45)	3 (75)
Hemoglobinopathies (%)	3 (14)	11 (35)	1 (25)
Bone marrow failure (%)	4 (14)	6 (19)	0 (0)
Donor			
MSD (%)	8 (38)	13 (42)	1 (25)
MUD (%)	12 (57)	13 (42)	2 (50)
MMFD/Haplo (%)	1 (5)	5 (16)	1 (25)
Pubertal status at HSCT			
Prepubertal (%)	20 (95)	20 (65)	4 (100)
(Post)pubertal (%)	1 (5)	11 (35)	0 (0)
Combining agents			
Fludarabine (%)	5 (24)	5 (16)	0 (0)
Fludarabine + Thiotepa/Melphalan (%)	0 (0)	5 (16)	1 (25)
Cyclophosphamide(%)	12 (57)	12 (39)	3 (75)
Cyclophosphamide + Melphalan/Etoposide/ Flu (%)	4 (19)	9 (29)	0 (0)
Exposure measured (%)	17 (81)	21 (68)	3 (75)
Low (<70 mg*h/L) (%)	11 (52)	15 (48)	1 (25)
High (≥70 mg*h/L) (%)	6 (29)	6 (20)	2 (50)

MSD: matched sibling donor, MUD: matched unrelated donor, MMFD: mismatched family donor.

Gonadal dysfunction in TREO-treated patients

At time of HSCT, 15 (47%) of 32 patients were prepubertal and 17 (53%) were (post) pubertal (Table 3). Median age at HSCT was 13.5 years (IQR 8.7-15.0) and median age at last visit was 16.6 years (IQR 15.3-18.6). Gonadal dysfunction occurred in 9 (28%) patients, 3 male and 6 female patients. In 5 patients (3 male, 2 female), gonadal dysfunction was transient and 3 patients (1 male, 2 female) needed temporary hormonal substitution. TREO exposure data was available of 19 patients. Lower TREO exposure (<1750 mg*h/L on day 1) was not associated with a reduced risk of gonadal dysfunction (OR 1.6 95%CI 0.16-36.6, p=0.71). The distribution of TREO exposure in relation to gonadal function is shown in Figure 1B.

Table 3. Gonadal dysfunction in TREO-treated patients

	No (N = 23)	**Yes (N=4)**	**Transient (N=5)**
Characteristic			
Sex (n: M/F)	13/50	0/4	3/2
Age (years, median (IQR))	11.5 (8.3-14.1)	15.9 (12.3-16.7)	14.9 (14.3-15.7)
Age at last follow up (years, median (IQR))	16.3 (14.5-18.3)	17.2 (15.5-19.0)	18.9 (17.4-21.5)
Length of follow up (years, median (IQR))	5.1 (2.7-8.3)	2.4 (1.7-4.9)	3.5 (3.3-6.6)
Diagnosis for HSCT			
Inborn errors of immunity (%)	4 (17)	0 (0)	1 (20)
Hemoglobinopathies (%)	17 (74)	3 (75)	4 (80)
Bone marrow failure (%)	2 (9)	1 (25)	0 (0)
Donor			
MSD (%)	13 (57)	1 (25)	1 (20)
MUD (%)	6 (26)	3 (75)	4 (80)
MMFD/Haplo (%)	3 (13)	0 (0)	0 (0)
ORD (%)	1 (4)	0 (0)	0 (0)
Pubertal status at HSCT			
Prepubertal (%)	13 (57)	1 (25)	0 (0)
(Post)pubertal (%)	9 (43)	3 (74)	5 (100)
Combining agents			
Fludarabine (%)	8 (35)	0 (0)	0 (0)
Fludarabine + Thiotepa/Melphalan (%)	12 (52)	3 (75)	4 (80)
Cyclophosphamide + Melphalan/Etoposide/Flu (%)	3 (13)	1 (25)	1 (20)
Exposure measured (%)	14 (61)	2 (50)	3 (60)
Low (<1750 mg*h/L) (%)	4 (17)	0 (0)	1 (20)
High (≥1750 mg*h/L) (%)	10 (43)	2 (50)	2 (40)

MSD: matched sibling donor, MUD: matched unrelated donor, MMFD: mismatched family donor, ORD: other related donor.

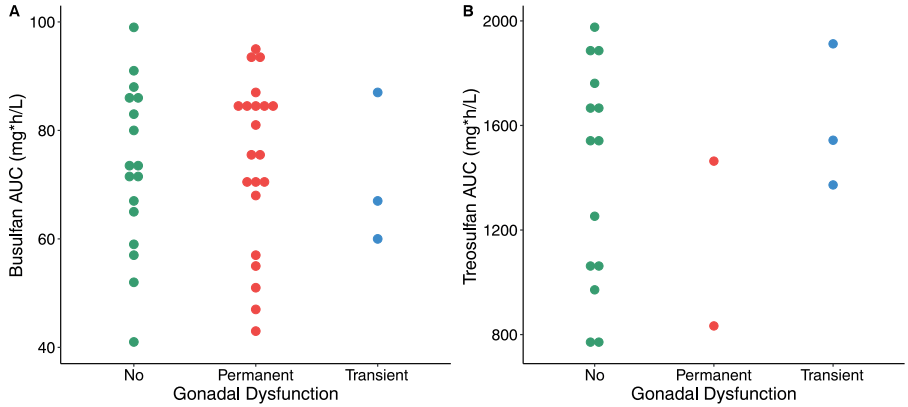

Figure 1. A) Busulfan cumulative exposure and B) treosulfan day 1 exposure in relation to gonadal dysfunction.

DISCUSSION

Both busulfan- and treosulfan-based conditioning have been demonstrated to be effective in patients transplanted for a nonmalignant disease [2, 15, 16]. With an increasing number of transplant survivors, the late effects of the transplant procedure become more important. We studied a cohort of 157 patients, transplanted for a nonmalignant disease, conditioned with a BU- or TREO-based regimen. Previous studies looked at different conditioning regimens in relation to gonadal function, pointing to a more favorable outcome in TREO-based conditioning [6, 17-19]. However, these studies did not take BU and TREO exposure into account. To the best of our knowledge, this is the first time BU and TREO exposure was studied in relation to late endocrine complications. While low BU exposure (reduced intensity conditioning) is known to result in less acute toxicity, it was not associated with a lower risk of gonadal dysfunction as compared to high exposure (myeloablative conditioning). Combining BU with either cyclophosphamide or fludarabine made no difference for the occurrence of gonadal insufficiency. Together, our data do not support the hypothesis that the use of reduced intensity BU-based conditioning lowers

the risk of gonadal dysfunction compared to high dose regimens. Gonadal dysfunction occurred at a much lower frequency in the TREO group in comparison to the BU group. No evidence was found for a correlation between TREO exposure and gonadal dysfunction, but numbers in our study were low, therefore probably lacking statistical power. Various studies in a variety of nonmalignant and malignant diseases have indicated that BU and TREO-based conditioning in general result in similar overall and event-free survival [20-23]. While reduced intensity BU-based conditioning has been reported to be beneficial in patients with co-morbidity to limit acute toxicity and improve outcome, our data do not support the premise that low exposure also lowers the risk for gonadal toxicity, either permanent or transient [24, 25]. Similarly, although the a priori risk of gonadal dysfunction is lower compared to BU-based conditioning, our data indicate that it is unlikely that TDM-based reduced treosulfan exposure will further reduce the risk for gonadal dysfunction.

Our study has some limitations. While the initial cohort consisted of 157 patients, the number of evaluable patients was lower, because a subgroup of patients was still prepubertal and were therefore not available for evaluation. Also, exposure data was not available for every patient. Future research should preferably be conducted in larger groups of former HSCT patients reaching adolescence and adulthood, so that other covariates, such as age at HSCT, sex and underlying condition can also be taken into account.

To conclude, in this first study on the association between BU- and TREO exposure and gonadal dysfunction after HSCT for nonmalignant diseases in childhood we demonstrate a higher incidence of gonadal dysfunction in the BU-conditioned group while no correlation was found with either BU or TREO exposure.

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