Treosulfan pharmacokinetics and dynamics in pediatric allogeneic stem cell transplantation

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CHAPTER 01

GENERAL INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established curative treatment for malignant and nonmalignant diseases in both adult and pediatric patients. It involves eradicating the patient's hematopoietic stem cells (HSCs) and replacing them with HSCs from a healthy donor.

If a patient is deemed eligible for HSCT - based on indication, medical history and pre-transplantation tests - the search for a potential donor begins. The outcome of HSCT depends partly on the match between donor and patient for the human leukocyte antigens (HLA) in the major histocompatibility complex (MHC), which are encoded by a group of genes on chromosome 6, belonging to MHC class I (HLA-A, HLA-B, HLA-C) and MHC class II (HLA-DR, HLA-DQ and HLA-DP) [1]. A set of HLA genes, called haplotype, is inherited from each parent, which makes the probability for two siblings to inherit the same genes (haplotypes) 25%. These siblings are genotypically HLA identical and this is generally considered to be the optimal donor-recipient combination. That is why the search for a suitable stem cell donor usually begins within the family of the patient. If there is no eligible donor in the family, the search continues for an unrelated donor. This search for an unrelated donor is usually based on high-resolution DNA typing for 10 alleles (A,B,C, DR and DQ), while in some cases additional DP matching is included. In most cases, a 10/10 HLA identical unrelated donor is considered the second best choice in the absence of a geno-identical family donor. If there are no matched unrelated donors available, the search is expanded to mismatched (9/10 HLA identical) or mismatched family (haplo) donors. In case of a mismatched donor, T-cell depletion *ex vivo* (e.g. TCRα/β depletion) or *in vivo* with post-transplant cyclophosphamide is often deployed [2]. If there are multiple eligible donors, other factors besides HLA matching play a role, such as donor age and sex, CMV status and blood (blood group/ABO match) type. The stem cell sources for HSCT are bone marrow (BM), mobilized peripheral blood stem cells (PBSC) and cord blood. The preferred stem cell source depends on a variety of patient and donor-related factors, such as age of the donor and recipient, underlying disease, manipulation of the graft, but is also dependent on the experience with these approaches in the transplantation center. Once a suitable donor is selected, the transplantation course can be planned.

In order to eradicate the stem cells of the patient, high-intensity chemotherapy (with or without total body irradiation (TBI)) is given, the so-called conditioning regimen. Depending on the underlying disease, the conditioning regimen usually consists of agents that have myeloablative (MA) properties to create 'space' in the bone marrow of the patient and to eradicate the primary disease [3]. Immunoablative/-suppressive agents are applied to prevent rejection (host-versus-graft) as well as graft-versus-host disease (GvHD). After conditioning, the stem cell graft of the donor is infused and the cells migrate to the bone marrow niches to produce new blood cells, which is called 'engraftment'. After engraftment, immune and hematological recovery occurs; a gradual process that can take several months. **Figure 1** gives an overview of the HSCT procedure.

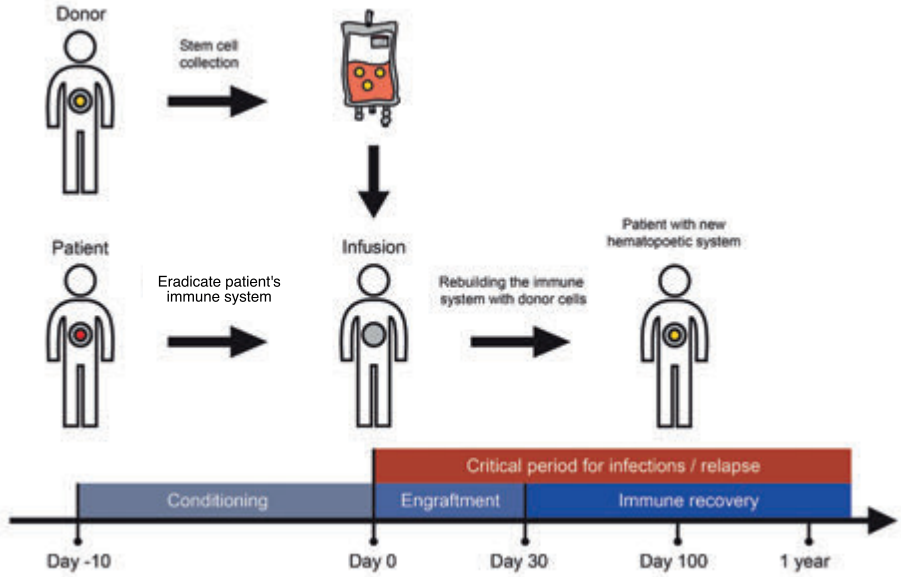

Figure 1: Overview of the allogeneic HSCT procedure.

INTENSITY OF CONDITIONING

Conditioning regimens are commonly defined as myeloablative, reduced intensity or minimal intensity. Although full consensus has not been reached, generally accepted definitions of these three types of regimens are as follows [2-4]:

Myeloablative conditioning (MAC): A combination of agents expected to produce profound aplasia and likely resulting in full donor chimerism, a situation where the newly developed hematopoietic system is of donor origin only [5]. Typical MAC protocols are based on high dose TBI, high dose busulfan or treosulfan (**Figure 2**).

Reduced intensity conditioning (RIC): Regimens containing reduced doses of myeloablative drugs (or radiotherapy), which are therefore less likely to achieve marrow ablation and more likely to produce mixed chimerism, a state where donor and recipient haematopoiesis coexist within the recipient. Examples are reduced busulfan with fludarabine or treosulfan with fludarabine, without thiotepa.

Minimal intensity conditioning (MIC): A regimen that causes minimal cytopenia and little early toxicity and can theoretically be given without stem cell support. In vulnerable patients MIC regimens are used to carefully eliminate their own bone marrow, followed by the infusion of donor HSCs. These regimens are mainly immunoablative. An example is fludarabine with low dose cyclophosphamide.

Figure 2 shows a classification of conditioning regimens, based on intensity and toxicity with some examples of common regimens.

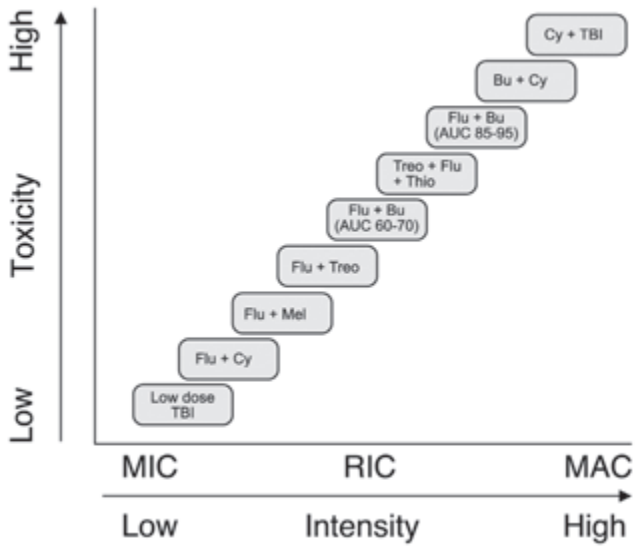

Figure 2: Classification of conditioning regimens, based on intensity (miminal intensity (MIC), reduced intensity (RIC) and myeloablative (MAC)) and toxicity. Bu: busulfan, Cy: cyclophosphamide, Flu: fludarabine, Mel: melphalan, TBI: total body irradiation, Thio: Thiotepa, Treo: treosulfan.

CHOICE OF CONDITIONING

The choice for the optimal conditioning regimen is dependent on different factors. To overcome rejection and ensure stable engraftment, the intensity of conditioning is traditionally higher in patients receiving transplants of unrelated and mismatched donors than when a transplant from a matched sibling is received. Serotherapy with either anti thymocyte globulin (ATG), anti-T lymphocyte globulin (ATLG) or alemtuzumab (Campath) is often added to the regimen to reduce the risk of GvHD and rejection. The underlying disease is also a factor that has to be taken into consideration. For nonmalignant diseases, less intense protocols can be sufficient to ensure engraftment, while reducing the risk of early (and possibly late) toxicity and GvHD. Other factors such as age, comorbidities and organ-specific toxicity risk

may determine the choice of the regimen. Especially in children, the influence of the conditioning regimen on growth and puberty (development) is an important aspect that has to be taken into account.

Historically, conditioning protocols were more myeloablative in nature. These regimens were associated with significant organ- and transplant-related toxicity and mortality [6]. Improvements have been made over the last decades to reduce transplant-related toxicity and mortality when using MAC. A number of pharmacological aspects, such as improvements in formulation and administration, more insight in the pharmacokinetic behavior of conditioning agents and increased availability of analytic tools have made an important contribution to these improvements. This is most explicitly illustrated with the alkylating agent busulfan.

In the early days, busulfan was only available as an oral formulation. Uniform dosing resulted in huge interindividual differences in exposure due to variation in absorption. Intravenous (i.v.) busulfan has widely replaced oral busulfan when this formulation became available, which reduced pharmacokinetic variability [7]. However, interpatient variability in clearance of i.v. busulfan is still reported to be up to 30% [8, 9]. Population pharmacokinetic studies were conducted to search for factors explaining this interpatient variability, which were age, body weight and GSTA1 genotype, among others [10]. Studies showed that a high area under the curve (AUC) of busulfan plasma concentration increased the risk of toxicity, while low busulfan concentrations may be associated with a higher risk of graft rejection and relapse [11]. Monitoring of busulfan levels and dose adjustments allowed for better control of the dose administered and reduction of the above mentioned risks; a clinical practice that is known as Therapeutic Drug Monitoring (TDM) [12]. With the introduction of intravenous busulfan, with more predictable pharmacokinetics (PK), tight control of plasma levels could be achieved and busulfan-mediated toxicity and mortality could be significantly reduced [13].

Unfortunately, not all (severe) toxicities can be avoided and long-term effects, such as infertility, alopecia and pulmonary diseases are still a problem in patients conditioned with busulfan [14-17]. Especially in children, these long-term effects can have a substantial

negative impact on quality of life. Reduced-intensity conditioning can be deployed to reduce transplant-related mortality (TRM), but the risk of relapse and graft failure or mixed chimerism may be increased [18]. Reducing toxicity without compromising HSCT efficacy could be of significant benefit. Replacing cyclophosphamide with fludarabine was an approach to reduce toxicity and demonstrated a significant reduction of TRM compared to busulfan with cyclophosphamide in patients with acute myeloid leukemia (AML) with no difference in relapse incidence [19]. Another strategy to reduce toxicity was replacing busulfan with treosulfan [20].

The research presented in this thesis focuses on the alkylating agent treosulfan.

TREOSULFAN

Treosulfan (L-threitol 1,4-bismethanesulphonate; dihydroxybusulfan) is a structural analogue of busulfan. It is a prodrug and a water-soluble alkylating agent. It is non-enzymatically and pH-dependently converted into a monoepoxide-(S,S-EBDM) and a diepoxide-derivative (S,S-DEB), in two consecutive reactions of intramolecular nucleophilic substitution (**Figure 3**). These epoxides are thought to be responsible for DNA alkylation, interstrand DNA crosslinking, chromosomal aberration and induction of apoptosis [21-23]. Treosulfan is originally used in oncology and approved for the treatment of ovarian carcinoma in most European countries [24]. Conventional doses are 5-8 g/m^2 every 3-4 weeks.

In preclinical studies, treosulfan has been shown to cause rapid and profound myelosuppression. In addition, it has more potent immunosuppressive characteristics than busulfan or cyclophosphamide [25]. In several clinical studies, a conditioning regimen containing Treo and Flu (with or without thiotepa) has been reported to result in rapid engraftment and complete donor chimerism. In addition, regimen related toxicity was low, as well as acute GvHD rates and transplant related mortality [26-32].

In contrast to busulfan, only a few studies were performed to investigate the pharmacokinetics of treosulfan in pediatric patients. These studies have shown great interpatient variability

in treosulfan exposure [33-36]. Factors that cause these great interpatient variability have not been investigated. Because clinical outcome of HSCT is associated with busulfan exposure, we hypothesize that treosulfan exposure might also be associated with clinical outcome. Also, treosulfan is relatively new in the field of HSCT and knowledge of acute and late side effects using treosulfan in the setting of HSCT (30-42 g/m^2) is limited.

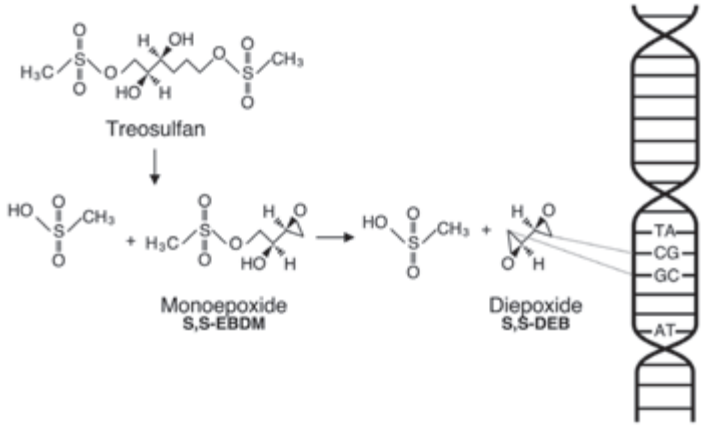

Figure 3. The conversion of treosulfan to the monoepoxide (S,S-EBDM) and the diepoxide (S,S-DEB), which causes interstrand DNA crosslinking.

AIMS AND OUTLINE OF THIS THESIS

This thesis focuses on treosulfan in the setting of pediatric stem cell transplantation. More specifically the aims of the thesis are:

- To investigate the pharmacokinetic behavior of treosulfan and develop a population PK model.
- To investigate the relationship between treosulfan exposure, early toxicity and clinical outcome.
- To acquire knowledge about the acute and late side effects.

Chapter 2 describes the development of a population PK model of treosulfan in a large cohort of pediatric patients undergoing HSCT and the exploration of covariates that are of possible influence on treosulfan pharmacokinetics. This population PK model is used to calculate treosulfan 'Area under the Concentration curve' (AUC) as a representation of treosulfan exposure.

Chapter 3 focuses on the relationship between treosulfan exposure and early toxicity in a multicenter pediatric cohort, while the focus of **Chapter 4** is more on the relationship between treosulfan exposure and clinical outcome in a cohort of patients transplanted for a nonmalignant disease.

In **Chapter 5**, the incidence and severity of myalgia - a side effect of treosulfan that was not mentioned in the original Summary of Product Characteristics (SmPC) - was identified using an electronic health record text mining tool and described in a cohort of patients that received treosulfan and compared to a cohort that received busulfan. **Chapter 6** describes the influence of busulfan and treosulfan exposure on long-term endocrine outcome, in particular gonadal function.

This thesis concludes with a review summarizing and discussing the evidence for TDM of the most commonly used conditioning agents in pediatric HSCT in **Chapter 7** and a discussion with future perspectives in **Chapter 8**.

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