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Brief Articles

Treosulfan-Thiotepa-Fludarabine–Based Conditioning Regimen for Allogeneic Transplantation in Patients with Thalassemia Major: A Single-Center Experience from North India

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A B S T R A C T

Hematopoietic stem cell transplantation (HSCT) is the definite treatment for patients with thalassemia major. A busulfan (Bu) and cyclophosphamide (Cy)–based regimen has been the standard myeloablative chemotherapy, but it is associated with higher treatment-related toxicity, particularly in patients classified as high risk by the Pesaro criteria. Treosulfan-based conditioning regimens have been found to be equally effective and less toxic. Consequently, we analyzed the safety and efficacy of treosulfan/thiotepa/fludarabine (treo/thio/flu)-based conditioning regimens for allogeneic HSCT in patients with thalassemia major between February 2010 and September 2012. We compared those results retrospectively with results in patients who underwent previous HSCT with a Bu/Cy/antithymocyte globulin (ATG)–based conditioning regimen. A treo/thio/flu-based conditioning regimen was used in 28 consecutive patients with thalassemia major. The median patient age was 9.7 years (range, 2-18 years), and the mean $CD34^+$ stem cell dose was 6.18×10^6/kg. Neutrophil and platelet engraftment occurred at a median of 15 days (range, 12-23 days) and 21 days (range, 14-34 days), respectively. Three patients developed veno-occlusive disease, 4 patients developed acute graft-versus-host disease (GVHD), and 2 patients had chronic GVHD. Treatment-related mortality (TRM) was 21.4%. Two patients experienced secondary graft rejection. We compared these results with results in patients who underwent previous HSCT using a Bu/Cy/ATG-based conditioning regimen. Twelve patients were treated with this protocol, at a median age of 7.2 years (range, 2-11 years). One patient had moderate veno-occlusive disease, 2 patients developed acute GVHD, 2 patients had chronic GVHD, and 2 patients experienced graft rejection. There was no TRM in this group. We found no significant differences between the 2 groups (treo/thio/flu vs Bu/Cy/ATG) in terms of the incidence of acute GVHD, chronic GVHD, TRM, and graft failure, although a trend toward higher TRM was seen with the treo/thio/flu regimen.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole curative treatment for patients with thalassemia major (TM) [1-3]. However, the clinical outcome after HSCT in children with TM who are classified as Pesaro class 3 and in adults with poor performance status and/or organ dysfunction remains unsatisfactory, owing to the high risk of treatment-related complications or graft failure [2-5], and the event-free survival rate is only 60% [5,6]. Even class 3 represents a heterogenous group of patients with overall survival after HSCT, varying from 39.01% in high-risk class 3 patients to 78.3% in other class 3 patients [7]. The most commonly used conditioning regimens incorporate busulfan (Bu), cyclophosphamide (Cy), and antithymocyte globulin (ATG) and carry a high rate of regimen-related toxicity. New treatment strategies have been shown to improve outcomes in high-risk patients [5,6]. In patients with class 3 thalassemia aged <17 years, the protocol 26 regimen (with hydroxyurea, azathioprine, and fludarabine added to Bu and Cy) was well tolerated, with 93% survival, and the incidence of post-HSCT graft failure decreased from 30% to 8% [8]. Low liver toxicity has also been observed with the use of i.v. busulfan in children with thalassemia undergoing HSCT [9].

Bernardo et al. [10] reported that treosulfan-based conditioning regimens are safe and effective in patients with thalassemia major. To minimize regimen-related toxicity, we treated patients with thalassemia major with a treosulfan/thiotepa/fludarabine (treo/thio/flu)-based conditioning regimen in a prospective manner and compared the results retrospectively with those in the patients treated earlier with a Bu/Cy/ATG- based regimen at the same center.

Study Design

This study involved an analysis of 28 consecutive patients with thalassemia major who underwent HLA-matched allogeneic HSCT using a treo/thio/flu-based conditioning

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Table 1
Characteristics of Patients Undergoing HSCT with Bu/Cy/ATG-Based and Treo/Thio/Flu-Based Conditioning Regimens

Characteristic	Bu/Cy/ATG (n = 12)	Thio/Treo/Flu (n = 28)	*P* value
Sex, n			.68
Male	8	15	
Female	4	13	
Age, y, median (range)	7 (2-11)	9.6 (2-18)	
Pesaro class, n			.24
Class 1	1	0	
Class 2	4	7	
Class 3	7	21	
$CD34^+$ cells, $\times 10^6$/kg, mean	7.61	6.76	.32
VOD, n	1	3	.82
Neutrophil engraftment, days, mean	16	15	.87
Acute GVHD, n	2	4	.77
Chronc GVHD, n	1	2	.66
Graft failure, n	2	2	.73
Death, n	0	6	.07

regimen at the Bone Marrow Transplant Centre, BLK Superspeciality Hospital, New Delhi, between February 2010 and September 2012. Data for 12 patients who underwent HSCT using a Bu/Cy/ATG-based regimen were also analyzed retrospectively and compared with the patients receiving a treo/thio/flu-based protocol. Informed consent from parent/guardian was obtained before a patient was enrolled. The study was approved by the hospital's Institutional Review Board and Ethical Committee.

All patients in the treo/thio/flu group received the same conditioning regimen, comprising i.v. thiotepa 8 mg/kg on day -6, treosulfan 14 g/m^2/day on day -5 to day -3, and fludarabine 40 mg/m^2/day on day -5 to day -2. Patients in the Bu/Cy/ATG group received oral busulfan 3.5 mg/kg/day on day -9 to day -6, cyclophosphamide 50 mg/kg/day on day -5 to day -2, and ATG 30 mg/kg/day on day -4 to day -2. Graft-versus-host disease (GVHD) prophylaxis included cyclosporine (2.5 mg/kg i.v. twice daily) and methotrexate (10 mg/m^2 i.v. on day +1 and 7 mg/m^2 on days +3, +6, and +11). All patients received cyclosporine for 9 to 12 months post-HSCT, with trough plasma cyclosporine levels maintained at 200 to 350 ng/mL. Acute and chronic GVHD were diagnosed and graded according to the Seattle criteria. Veno-occlusive disease (VOD), also called *sinusoidal obstruction syndrome*, was diagnosed according to clinical criteria as the presence of 2 of the following before day 21 post-HSCT: (1) hyperbilirubinemia (bilirubin >2.0 mg/dL), (2) painful hepatomegaly, and (3) unexplained weight gain (>2% from baseline). Severity of VOD was classified as mild, moderate, or severe. Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9$/L and a platelet count $\geq 20 \times 10^9$/L unsupported for 7 days. Chimerism was evaluated by fluorescein in situ hybridization in sex-mismatched transplantations and by PCR in others.

Statistical Analysis

The primary study endpoints were to determine the incidences of graft failure and treatment-related mortality (TRM) after HSCT. Secondary endpoints included the incidence and severity of VOD and acute and chronic GVHD. The incidences of relapse, TRM, and GVHD were calculated using cumulative incidence estimates. The differences in outcomes between the 2 groups (treo/thio/flu group and Bu/Cy/ATG group) were analyzed as well. Fisher's exact test was used for discrete variables, and the *t*-test was used for continuous variables. The log-rank test was used for the difference in survival outcome between the 2 groups. A *P* value $< .05$ was considered statistically significant.

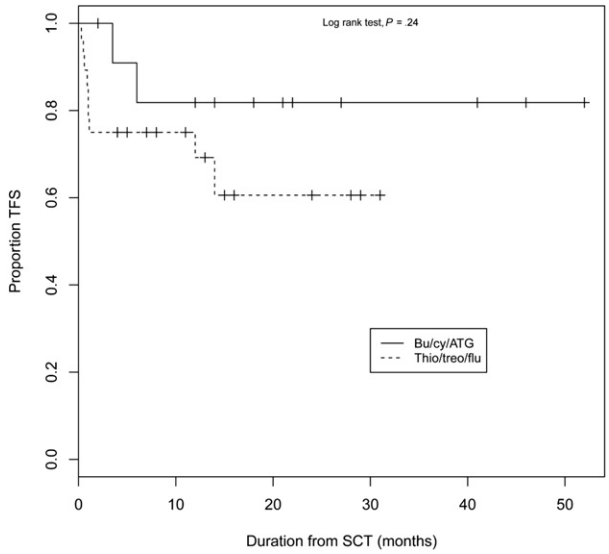

Figure 1. Thalassemia-free survival by conditioning regimen.

RESULTS

Twenty-eight consecutive patients with thalassemia major who underwent allogeneic HSCT using a treo/thio/flu-based conditioning regimen were evaluated. The median patient age was 9.6 years (range, 2-18 years). The group included 15 males and 13 females. Patients were classifed according to the Pesaro classification scheme based on liver size, adequacy of chelation, and hepatic fibrosis. Seven patients were in Pesaro class 2, and 21 patients were in Pesaro class 3. When stratified according to age (≥7 years) and liver size (≥5 cm) [7], 11 of 21 class 3 patients (52.4%) were considered high risk. The stem cell source was filgrastim (granulocyte colony-stimulating factor)-mobilized peripheral blood in 2 patients, bone marrow in 21 patients, and bone marrow plus cord blood in 5 patients. The mean CD 34^+ stem cell dose was 6.18×10^6/kg. Neutrophils and platelets engrafted at a median of 15 days (range, 12-23 days) and 21 days (range, 14-34 days), respectively. Three patients had a major blood group mismatch, 2 patients had a bidirectional blood group mismatch, and 4 patients had a minor blood group mismatch. The median duration of follow-up was 387 days (range, 37-930 days). Nineteen patients developed World Health Organization stage 1-3 oral mucositis (stage 3 in 2 patients). Only 3 patients required total parenteral nutrition, 2 with stage IV gut GVHD and 1 with stage 3 oral mucositis. No renal, pulmonary, cardiac, or central nervous system toxicities were observed. Four patients developed grade II-IV acute GVHD, for a cumulative incidence of 14.3% (95% confidence interval [CI], 3.6%-26.2%). Two patients had grade II-IV skin GVHD, and 2 patients had grade II-IV gut GVHD. Three patients developed severe VOD. Six deaths occurred in the treo/thio/flu group, including 3 due to VOD (on days +17, +27, and +29), 2 due to acute gut GVHD (on days +37 and +60), and 1 due to sepsis, for a cumulative incidence of TRM of 21.4% (95% CI, 4%-35.8%). The cumulative incidence of limited chronic GVHD was 10% (95% CI, 1%-16%). The median hospital stay was 37 days