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Total Body Irradiation and Cyclophosphamide Plus Antithymocyte Globulin Regimen Is Well Tolerated and Promotes Stable Engraftment as a Preparative Regimen before T Cell–Replete Haploidentical Transplantation for Acute Leukemia

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A B S T R A C T

We compared total body irradiation (TBI, 700 cGy)/cyclophosphamide (Cy, 3.6 g/m^2)/simustine (250 mg/m^2) plus antithymocyte globulin (ATG) (TBI/Cy plus ATG) with cytarabine (8 g/m^2)/i.v. busulfan (Bu, 9.6 mg/kg)/Cy (3.6 g/m^2)/simustine (250 mg/m^2) plus ATG (modified Bu/Cy plus ATG) as preparative therapy in T cell–replete haploidentical hematopoietic stem cell transplantation (haplo-HSCT) for acute leukemia. From August 2009 to August 2013, 38 consecutive patients using TBI/Cy plus ATG regimen for T cell–replete haplo-HSCT (TBI group) at our center were eligible, which contained 28 high-risk and 10 standard-risk patients. A nested case-control study was designed. Seventy-seven patients using modified Bu/Cy plus ATG regimen (Bu group) were randomly selected in a 1 to 3:1 ratio matching for age, disease and status, year of HSCT (±2 years), and length of follow-up. Only 1 graft failure occurred in the TBI group. The incidence and time of neutrophil and platelet engraftment were comparable between the 2 groups. Severe grades III/IV graft-versus-host disease was observed in 13.4% of Bu group and only 2.6% of TBI group ($P = .083$). More toxicity of the liver (37.7% versus 10.5%; $P = .002$) and more hemorrhagic cystitis occurred in the Bu group (49.3% versus 23.7%, $P = .008$). Diarrhea was more common in the TBI group (44.7% versus 22.1%; $P = .031$). No significant differences were found in the 2-year incidences of relapse (26.5% for TBI group versus 32.3% for Bu group, $P = .742$), 1-year transplant-related mortality (12.6% versus 16.2%, $P = .862$), 2-year overall survival (60.2% versus 57.0%, $P = .937$), and 2-year incidence of disease-free survival (57.9% versus 56.6%, $P = .845$) between the 2 groups. We conclude that the TBI/Cy plus ATG regimen seems to be feasible in T cell–replete haplo-HSCT, which promotes stable engraftment and a lower incidence of liver toxicity and hemorrhagic cystitis. However, longer follow-up is necessary to determine the late relapse rate and late toxicity.

INTRODUCTION

Advances in haploidentical transplantation have allowed for the use of hematopoietic stem cell transplantation (HSCT) in many patients without an HLA-identical donor or who urgently require transplantation [1-7]. Busulfan (Bu) in combination with cyclophosphamide (Bu/Cy) is the most frequently used myeloablative preparative regimen in T cell–replete haploidentical HSCT (haplo-HSCT), and encouraging results have been reported [3-5,8,9]. However, for patients with high-risk hematological malignancies or T cell acute lymphoblastic leukemia (T-ALL) beyond the first complete remission (CR1), especially with advanced diseases, using the Bu/Cy regimen, the cure rate with haploidentical transplantation has not been satisfactory due to high rates of disease relapse and transplant-related mortality (TRM), which prompted us to attempt a new pretransplantation regimen for these patients.

Total body irradiation (TBI) in combination with Cy is another commonly used myeloablative preparative regimen in sibling and unrelated donor transplantation. TBI can eradicate leukemic cells in "sanctuary sites" such as the central nervous system or the testicles. Problems of drug excretion or metabolism do not exist with TBI, unlike with Bu, which has some toxic effects that are rare with TBI, such as veno-occlusive disease of the liver, hemorrhagic cystitis, and so on. Many studies of sibling or unrelated donors transplantation have even found that the TBI/Cy regimen has some advantages in reducing relapse and TRM, especially for ALL and high-risk leukemia, compared with Bu-based regimens [10-13]. However, there remains a lack of data on haplo-HSCT using TBI/Cy regimen as myeloablative preparative regimen with a T cell–replete graft. Low-dose TBI has been used in nonmyeloablative preparative regimens for haplo-HSCT. Investigators from Johns Hopkins University designed a TBI-based nonmyeloablative preparative regimen consisting of fludarabine, TBI (2 Gy), and Cy pre- and post-transplantation followed by a T cell–replete (unmanipulated) bone marrow graft. This approach is effective for achieving donor engraftment and immunological tolerance [14]. These promising experiences of nonmyeloablative TBI-based regimens prompted us to investigate the feasibility of TBI-based myeloablative regimen in T cell–replete haplo-HSCT.

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In August 2009 we expanded the use of combination TBI and Cy plus antithymocyte globulin (ATG) to recipients of T cell–replete haplo-HSCT for high-risk leukemia or T-ALL. The aim of this study was to retrospectively evaluate whether the TBI/Cy plus ATG regimen was well tolerated and sufficiently immunosuppressive for engraftment in T cell–replete haplo-HSCT. A nested case-control study was designed to compare the transplant outcomes between patients using TBI/Cy plus ATG regimen with those using our conventional modified Bu/Cy plus ATG regimen in T cell–replete haplo-HSCT. The TBI/Cy plus ATG regimen may provide an alternative to patients who are not suitable candidates for Bu-based regimens.

METHODS

Patients and Control Subjects

From August 2009 to August 2013, 38 consecutive patients with acute leukemia who used a TBI/Cy plus ATG regimen (TBI group) as described below for T cell–replete haploidentical HSCT at our center were eligible. Disease status at transplantation was defined as "standard risk" if patients were in CR1 or second complete remission (CR2) of acute leukemia without the history of HSCT. Patients were categorized as "high risk" if they achieved CR after ≥3 course of chemotherapy [15], were in more than the third complete remission (CR3) of acute leukemia, were not in remission, and were in CR2 but with a history of HSCT (autologous or allo-HSCT). Among the 38 patients using TBI/Cy plus ATG regimen, 28 patients were classified as "high risk" and 10 patients as "standard risk." Although the risk category in this study was not according to cytogenetics, all 10 standard-risk patients were diagnosed with T-ALL in CR1 without any cytogenetic abnormalities, whereas adverse cytogenetic abnormalities were only seen in high-risk patients. Of the 28 high-risk patients, 4 patients had t(9;22) and 1 patient had t(4;11) abnormalities.

To choose control subjects using modified Bu/Cy plus ATG regimen as described below for T cell–replete haplo-HSCT, a nested case-control study was designed. Using "risk set sampling" [16], we randomly selected control subjects in a 1 to 3:1 ratio matching for age, disease and status (high-risk acute leukemia or T-ALL in CR1 without cytogenetic abnormalities), year of HSCT (±2 years), and length of follow-up. Finally, 77 matched control subjects using modified Bu/Cy plus ATG regimen (Bu group) were chosen for the analyses. Among them, 47 patients had high-risk leukemia and 30 patients were diagnosed with T-ALL in CR1 without cytogenetic abnormalities. Of the high-risk patients in the Bu group, 2 patients had t(9;22) and 1 patient t(4;11) abnormalities. The characteristics of TBI patients and control subjects are summarized in Table 1. The ethics committee of the Peking University People's Hospital approved the study protocol. Informed consent was obtained according to the Declaration of Helsinki.

Table 1
Patient Characteristics

Variable	TBI Group (n = 38)	Bu Group (n = 77)	*P*
Median age (range)	20 (13-46)	24 (8-51)	.283
Gender			.090
Male	30 (78.9)	48 (62.3)	
Female	8 (21.1)	29 (37.7)	
HSCT ≥ 7 mo from diagnosis	25 (65.8)	40 (51.9)	.169
Diagnosis			.030
AML	6 (15.8)	28 (36.4)	
ALL	32 (84.2)	49 (63.6)	
Disease status			
Standard risk (T-ALL in CR1)	10 (26.3)	30 (39.0)	.215
High risk	28 (73.4)	47 (61.0)	
ALL	22 (57.9)	19 (24.7)	.002
To achieve CR after ≥3 courses of chemotherapy	4 (10.5)	2 (2.6)	
≥CR3	0 (0)	3 (3.9)	
Not in remission	17 (44.8)	14 (18.2)	
CR2 with previous HSCT	1 (2.6)	0 (0)	
AML	6 (15.5)	28 (36.3)	
To achieve CR after ≥3 courses of chemotherapy	2 (5.3)	4 (5.2)	
≥CR3	1 (2.6)	4 (5.2)	
Not in remission	0 (0)	17 (22.1)	
CR2 with previous HSCT	3 (7.9)	3 (3.9)	
Sex donor/recipient			.060
Matched	19 (50.0)	43 (55.8)	
Mismatched	19 (50.0)	34 (44.2)	
HLA mismatched			.825
1 locus	4 (10.5)	7 (9.1)	
2 loci	13 (34.2)	22 (28.6)	
3 loci	21 (55.3)	48 (62.3)	
ABO blood type			1.000
Identical	23 (60.5)	46 (59.7)	
Nonidentical	15 (39.5)	31 (40.3)	
ATG dose			1.000
6 mg	11 (28.9)	22 (28.6)	
10 mg	27 (71.1)	55 (71.4)	
Median $CD34^+$ cells $\times 10^6$/kg (range)	2.55 (.67-5.89)	2.82 (1.04-6.72)	.277
Median MNC $\times 10^8$/kg (range)	7.85 (5.89-15.43)	7.85 (5.08-14.5)	.749
Prophylactic DLI	15 (39.5)	35 (45.5)	.697

AML indicates acute myelocytic leukemia; MNC, mononuclear cell count. Values are number of incidences with percents in parentheses unless otherwise noted.

Conditioning Regimen

Patients in the TBI group received conditioning with TBI (770 cGy) with particle shielding of the lungs (600 cGy) on day −6, and patients in the Bu group received cytarabine (4 g/m^2 i.v. per day) on days −10 to −9, Bu (3.2 mg/kg i.v. per day) on days −8 to −6, both followed by Cy (1.8 g/m^2 i.v. per day) on days −5 and −4, simustine (Me-CCNU; 250 mg/m^2 p.o.) on day −3, and rabbit ATG (Sang Stat, Lyon, France) on days −5 to −2.

The total dose of ATG was 10 mg/kg for patients with T-ALL in CR1 in both TBI and Bu groups. For high-risk patients in both groups, the total dose of ATG was 6 or 10 mg/kg. The total dose of ATG was administered intravenously over 10 hours in 4 divided doses daily for 4 days (day −5 to −2).

Hyperhydration and alkalization were administered from day −6 until the last dose of Cy was given. During the 2 days when patients received Cy, mesna was given for hemorrhagic cystitis prophylaxis.

Stem Cell Graft Harvest

Donors were primed with recombinant human granulocyte colony-stimulating factor (filgrastim; Kirin, Japan; 5 to 10 μg/kg/d s.c.) for 5 to 6 consecutive days. Bone marrow was harvested on the fourth day (day 0), and peripheral blood stem cells were collected with a blood cell separator (Spectra LRS; Cobe BCT Inc, Lakewood, CO) on the fifth day (day 2) and on the sixth day (day 3) if needed.

Graft-Versus-Host Disease Prophylaxis and Treatment

All transplant recipients received cyclosporine A (CsA), mycophenolate mofetil, and short-term methotrexate for graft-versus-host disease (GVHD) prophylaxis. The primary dosage of CsA was 2.5 mg/kg/d i.v. from day −9 until bowel function returned to normal. At that point, patients were switched to oral CsA. Whole blood CsA concentration was monitored weekly using fluorescence polarization immunoassay, and the dosage was adjusted to a blood concentration of 150 to 250 ng/mL. When there was no evidence of GVHD by day +180, CsA dosage was reduced gradually. When GVHD did occur, CsA was continued. Mycophenolate mofetil was administered orally, .5 g every 12 hours, from day −9 to day +30, was tapered from .5 g every 12 hours to .25 g every 12 hours on day +30, and was discontinued over days +45 to +60. The dosage of methotrexate was 15 mg/m^2, administered i.v. on day +1, and 10 mg/m^2 on days +3, +5 and +11. Acute GVHD was treated with steroids (methylprednisolone .5 to 1 mg/kg/d). For inadequate or no response to primary therapy, anti-CD25 monoclonal antibodies (basiliximab; Novartis Pharma AG, Basle, Switzerland; or Zenapax; Hoffmann-La Roche Corp, Nutley, NJ) were administered to the patient. Chronic GVHD was treated with CsA and steroids.

Regimen-Related Toxicity

According to the World Health Organization toxicity scale [17], the incidence and severity of mucositis, diarrhea, fever, pain, hepatic, renal, and central nervous system toxicities were estimated from day −5 to day +15. The maximum score for each organ system was recorded.

Supportive Care

All patients were hospitalized in rooms with high-efficiency particle-arresting air filters and received prophylactic antibiotics and antifungals with oral trimethoprim-sulfamethoxazole and fluconazole starting on day −10. Ganciclovir (5 mg/kg) was administered i.v. twice daily from days −5 to −2. Patients were monitored weekly for cytomegalovirus (CMV) by DNA (real-time PCR). All patients with 2 consecutive tests positive for CMV-DNA were treated with ganciclovir at a standard dose of 5 mg/kg twice daily until the