Biol Blood Marrow Transplant 23 (2017) 1359–1366

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Ex Vivo Mesenchymal Precursor Cell–Expanded Cord Blood Transplantation after Reduced-Intensity Conditioning Regimens Improves Time to Neutrophil Recovery

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Article history:
Received 28 December 2016
Accepted 1 May 2017

Key Words:
Cord blood
Expansion
Reduced-intensity conditioning
Engraftment
Neutrophil recovery

A B S T R A C T

We previously showed the safety of using cord blood (CB) expanded ex vivo in cocultures with allogeneic mesenchymal precursor cells (MPC) after myeloablative conditioning with faster recovery of neutrophils and platelets compared with historical controls. Herein, we report the transplantation outcomes of 27 patients with hematologic cancers who received 1 CB unit expanded ex vivo with MPCs in addition to an unmanipulated CB (MPC group) after reduced-intensity conditioning (RIC). The results in this group were compared with 51 historical controls who received 2 unmanipulated CB units (control group). The analyses were stratified for 2 RIC treatment groups: (1) total body irradiation 200 cGy + cyclophosphamide + fludarabine) (TCF), and (2) fludarabine + melphalan (FM). Coculture of CB with MPCs led to an expansion of total nucleated cells by a median factor of 12 and of $CD34^+$ cells by a median factor of 49. In patients in whom engraftment occurred, the median time to neutrophil engraftment was 12 days in the MPC group, as compared with 16 days in controls ($P = .02$). The faster neutrophil engraftment was observed in both RIC groups. The cumulative incidence of neutrophil engraftment on day 26 was 75% with expansion versus 50% without expansion in patients who received FM as the RIC regimen ($P = .03$). Incidence of neutrophil engraftment was comparable in MPC and control groups if treated with TCF (82% versus 79%, $P = .40$). Transplantation of CB units expanded with MPCs is safe and effective with faster neutrophil engraftment even after RIC regimens.

INTRODUCTION

Cord blood (CB) transplantation (CBT) has historically been associated with prolonged time to engraftment, high risk of graft rejection, delayed immune reconstitution, and resultant infections, primarily related to the naivety of CB cells and low total cellular content in the graft as compared with bone marrow or peripheral blood progenitor cells graft [1-8]. In an attempt to improve the cell dose, several techniques have been devised to expand CB units ex vivo before infusion [9-13]. Our group also previously reported encouraging outcomes of double unit CBT (DCBT) where 1 of the CB units was expanded ex vivo by coculturing with mesenchymal precursor cells (MPC) and the second unit was infused unmanipulated [14]. Among patients who received the expanded CB unit, neutrophil engraftment occurred at a median of 15 days compared with 24 days in the historical controls. All patients in that study received myeloablative conditioning. However, high-dose myeloablative regimens are poorly tolerated by medically infirm or older individuals. The use of reduced-intensity conditioning (RIC) with CBT has been an effective strategy for older and less-fit patients with a potential curative intent [2,15-19] with a low treatment-related mortality [2,18,20-22].

Financial disclosure: See Acknowledgments on page 1365.

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http://dx.doi.org/10.1016/j.bbmt.2017.05.002
1083-8791/

Herein, we retrospectively studied the outcomes of adult patients with hematological malignancies who received RIC followed by infusion of an MPC-expanded CB unit plus an unmanipulated unit compared with outcomes of recipients of 2 unmanipulated CB units.

METHODS

Patient Population

We evaluated all consecutive adult patients with hematological malignancies who received RIC followed by DCBT where 1 CB unit was infused unmanipulated and the second unit was expanded ex vivo by coculturing it with MPCs before infusion (MPC group, n = 27), as described previously, [14] between January 2004 and January 2015. During the study period, the clinical trial with the use of 1 ex vivo–expanded CB unit in addition to a nonexpanded CB unit was a departmental priority for enrollment for all double CB patients eligible for RIC regimens. Major reasons for not enrolling into the clinical trial were not receiving insurance approval and/or patient and physician preferences. The group of adult patients who received the same RIC regimens as the study cohort followed by the infusion of 2 unmanipulated CB units during the same time period was defined as the *control cohort* (n = 51). Patients with prior allogeneic hematopoietic stem cell transplantation were excluded from the analysis. All patients underwent transplantation between January 2004 and January 2015.

Ex Vivo CB Expansion

The criteria for CB unit selection and the technique for their expansion with MPCs were previously reported [14]. Briefly, the CB unit with a lower total nucleated cell (TNC) dose was thawed 2 weeks before the planned CBT and cocultured with MPCs. On the day of transplantation, the unmanipulated cord was thawed, washed, and infused followed by the infusion of the MPC-expanded unit.

Conditioning Regimens and Graft-versus-Host Disease Prophylaxis

RIC regimens consisted of (1) fludarabine 40 mg/m^2 administered intravenously on days –6 to –2, cyclophosphamide 50 mg/kg intravenously on day –6, and 200cGy of total body irradiation on day –1 (TCF) or (2) fludarabine 40 mg/m^2 administered intravenously on days –5 to –2 and melphalan 140 mg/m^2 intravenously on day –2 (FM).

Graft-versus-host disease (GVHD) prophylaxis was provided with tacrolimus and mycophenolate mofetil. Tacrolimus .03 mg/kg continuous i.v. infusion daily was started on day –2, and the dose was adjusted to maintain serum trough levels between 5 ng/m and 15 ng/mL. The route was switched to oral dosing when tolerated and the taper was started around day +180 in the absence of GVHD. Mycophenolate mofetil 1 gm twice a day i.v. (or oral if tolerated) was started on day –3 and continued through day +100. Patients also received rabbit antithymocyte globulin 1.25 mg/kg i.v. on day –4 and 1.75 mg/kg i.v. on day –3.

Supportive Care

Filgrastim 5 μg/kg was administered subcutaneously daily starting day 0 until absolute neutrophil count was $>1000 \times 10^6$/L. Antimicrobial prophylaxis and blood products were administered according to the institutional guidelines.

Endpoints and Definitions

Our hypothesis was that the use of an MPC-expanded CB unit along with 1 unmanipulated CB unit would lead to faster time to neutrophil engraftment than use of 2 unmanipulated CB units after RIC. The primary study endpoint was the median time to *neutrophil engraftment*, defined as the first of 3 consecutive days after DCBT with an absolute neutrophil count of $\geq .5 \times 10^9$/L. Patients who had no neutrophil recovery by day 42 and had bone marrow cellularity <10% were considered to have *graft failure*. Serial sampling of the bone marrow and/or peripheral blood at days 30 and 100 after transplantation determined donor chimerism using 8 highly polymorphic microsatellite markers (purchased from Integrated DNA Technologies, Inc., Coralville, IA) in a multiplex polymerase chain reaction in recipient and donor units. It was determined that 1 unit was providing hematopoiesis solely if that unit contributed to more than 95% of the chimerism.

Other study endpoints included the median time to platelet engraftment, acute and chronic GVHD, nonrelapse mortality (NRM), disease-free survival (DFS), and overall survival (OS). *Time to platelet engraftment* was defined as the first of 7 consecutive days with a platelet count of $\geq 20 \times 10^9$/L without platelet transfusion support. Acute GVHD was assessed clinically as recommended [23] and the maximum overall grade was recorded. Chronic GVHD was recorded as limited or extensive [24]. *NRM* was defined as death without evidence of disease recurrence. *DFS* was defined as the time from DCBT to either death or relapse. *OS* was defined as the time from DCBT to death from any cause.

Patients with different hematological malignancies were categorized by Disease Risk Index defined by Armand et al. [25].

Statistical Analysis

Patient and transplantation characteristics in the MPC and control groups were compared using the Wilcoxon rank-sum test for continuous variables and the chi-square test or Fisher exact test for categorical variables. Time to neutrophil and platelet engraftment was estimated as the cumulative incidence function considering graft failure or early death as competing risks. The cumulative incidence of NRM was estimated considering disease progression or death due to disease as competing risks. The cumulative incidence of GVHD was estimated considering death or disease progression in the absence of GVHD as competing risks. Actuarial OS and DFS were calculated using the Kaplan-Meier method. Outcomes were compared using Cox's proportional hazards regression analysis. Statistical significance was established at the .05 level. Statistical analysis was performed using STATA 11.0 (StataCorp. 2009. Stata Statistical Software: Release 9. College Station, TX: StataCorp LP.)

RESULTS

Patient and disease characteristics of the comparison groups were similar except disease diagnoses, as presented in Table 1. Of 27 patients in MPC group, 18 (67%) had acute myeloid leukemia or myelodysplastic syndrome compared with 20 of 51 patients in the control group (39%) ($P = .03$). The median age at transplantation was similar with 59 (interquartile range, 49 to 67) in the MPC group and 57 (interquartile range, 48 to 63) in the control group, respectively ($P = .30$). Disease Risk Index [25] was high/very high in 9 (33%) and intermediate in 16 of 27 (59%) MPC patients compared with 8 (16%) and 38 (75%) in the control group, respectively ($P = .10$). More than one-half of the patients in the study cohort, 15 patients in the MPC group (56%) and 31 (61%) in the control group, had advanced disease beyond first or second complete remission at transplantation.

The RIC regimen used for transplantation was TCF in 11 MPC (41%) and 29 (57%) control group patients. The rest of the patients received FM as the conditioning regimen. The distribution of HLA matching at 4 loci (-A, -B, -C, and -DRB1) using high-resolution testing was similar between groups. The dominant unit was matched to the recipient at 5/8 to 6/8 allele level in 16 (59.3%) patients and in 28 (54.9%) patients of the MPC and control group, respectively ($P = .60$).

The median follow-up was 39 months (range, 12 to 86) in the MPC group and 22 months (range, 3 to 88) in the control group.

Coculture of CB Units with MPCs Led to an Increase in the TNC Dose and CD34$^+$ Cell Dose Infused

Coculture of CB units with MPCs led to an expansion of TNC by a median factor of 12 (range, 3 to 46.55) and that of CD34$^+$ cells by a median factor of 49 (range, 3.5 to 98.8) as shown in Figure 1. After expansion, the median TNC dose increased to 5.7×10^7/kg from a pre-expansion median dose of $.55 \times 10^7$/kg. Similarly, expanded units had higher CD34$^+$ cell dose, with a median of 16×10^5/kg compared with pre-expansion dose of $.3 \times 10^5$/kg.

This increase in the number of TNC and CD34$^+$ cells doses after CB expansion was associated with an increase in the total stem cell dose infused for the MPC group compared with the control group. Patients in the expanded group received a median TNC dose of 7.9×10^7/kg and CD34$^+$ cell dose of 19.7×10^5/kg compared with a median TNC dose of 4.25×10^7/kg and CD34$^+$ cell dose of 4.3×10^5/kg in the control group, respectively ($P < .001$ for both TNC and CD34$^+$).