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Low Rate of Infusional Toxicity following Expanded Cord Blood Transplantation

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Abstract

Umbilical cord blood (CB) is utilized with increasing frequency to restore hematopoiesis in bone marrow transplant patients lacking a suitable HLA-matched donor. CB transplantation is limited by low cell doses and delays in neutrophil and platelet engraftment. CB progenitors expanded ex vivo prior to transplantation provide more rapid hematopoietic and immune reconstitution, as well as less engraftment failure compared to unmanipulated CB. However, the safety of infusing double and ex vivo expanded CB has not been systematically examined. Here we review the immediate adverse events (AE) associated with the infusion of CB occurring within 24 hours in 137 patients enrolled in clinical CB transplant trials at the MD Anderson Cancer Center from February 2004 to May 2010. All patients received an unmanipulated CB unit followed by infusion of a second unmanipulated CB unit or a second CB unit expanded ex vivo using cytokines in a liquid culture system or in mesenchymal stromal cell co-cultures. A total of three Grade 2 and two Grade 3 infusion reactions occurred within 24 hours of CB transplantation. This resulted in an AE rate of 3.7%. The majority of AEs manifested as signs of hypertension. No association with

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patient age, sex, disease status, premedication, ABO compatibility or total infusion volume was observed. In summary, the incidence of infusion related toxicities in patients who receive unmanipulated and ex vivo-expanded double CB transplantation is low. We conclude that the infusion of unmanipulated followed by expanded CB products is a safe procedure associated with a low probability of inducing severe reactions.

Keywords

Cord blood transplantation; ex vivo expansion; cell culture

INTRODUCTION

Umbilical cord blood (CB) is utilized with increasing frequency to restore hematopoiesis in bone marrow transplant patients lacking a suitable human leukocyte antigen (HLA)-matched donor due both to its ease of procurement as well as a decreased incidence of GVHD in comparison with bone marrow transplantation. CB transplantation, however, is limited by low cell doses which results in delayed neutrophil and platelet engraftment, as well as high rates of engraftment failure.[1–5] The use of two CB grafts has become a standard practice for adult patients, providing a higher cell dose and less engraftment failure compared to single CB transplant recipients.[6–8] An alternative method to increase the total neutrophil count (TNC) is to expand CB progenitor cells ex vivo. The ex vivo expansion of CB prior to transplantation allows for the administration of higher cell doses and has been demonstrated to provide more rapid neutrophil and platelet engraftment as well as less engraftment failure compared to unmanipulated CB.[9–11]

There are a variety of methods currently under investigation for expanding CB ex vivo, which include static liquid cultures alone[9,12] or in conjunction with notch-ligand,[10] as well as stromal co-culture[11] and continuous perfusion culture systems.[13] CB culture in the presence of various proteins, such as Notch ligand or with MSC co-cultures greatly increase the number of CD34+ progenitor cells with repopulating ability and subsequently leads to more rapid myeloid engraftment.[10]

Although the adverse events (AE) associated with traditional stem cell transplants are well defined, the relative safety regarding the infusion of double and ex vivo expanded CB transplantation has not been widely reported. Here we review immediate AEs occurring within 24 hours of infusion among 137 patients receiving double CB transplantation with either two unmanipulated or one unmanipulated plus one expanded CB unit at MD Anderson Cancer Center between February 2004 to May 2010.

METHODS

Patients

All patients were treated on MDACC IRB approved protocols conducted under IND after approval by the FDA. This retrospective chart review was also approved by the IRBs at Baylor College of Medicine and MDACC.

All patients received either a myeloabative or non-myeloablative preparative regimen on days -8 through -2, followed by infusion of two CB units on Day 0. All patients were infused with a single unmanipulated CB unit followed by the immediate infusion of a second unit that was unmanipulated (n=48) or expanded ex vivo in either a liquid culture system (n=46)[14] or in MSC co-cultures (n=43).[11] Patients were premedicated with 25 mg intravenous diphenhydramine and 100 mg intravenous hydrocortisone before each CB unit infusion. Patients intolerant of diphenhydramine were pre-medicated with hydrocortisone alone.

Cord Blood Processing and Infusion

Unmanipulated CB units were thawed, washed with Dextran-40 and Human Serum Albumin (HSA) and infused. For cells expanded in liquid culture, $CD133^+$ cells were selected using the Miltenyi Clinimacs columns and cultured for 14 days in MEM-alpha medium (Hyclone) containing granulocyte colony-stimulating factor (G-SCF; Amgen, Thousand Oaks, CA), Stem Cell Growth Factor (SCF; Cellgenix, Freiburg, Germany), thrombopoietin (TPO; R&D), and Flt-3 ligand (Flt3-L; Cellgenix).[14] For cells grown with MSCs, MSC co-cultures were generated by culturing enriched CB mononuclear cells for 14 days on MSC monolayers in serum-free medium (CellGenix) containing G-SCF, SCF, Flt-3L and TPO.[11] On day 7, the non-adherent cells were transferred to a bag with additional media and growth factors while the flasks with the adherent cells were similarly re-fed. On culture day 14, all the cells from the bags and flasks were combined, washed, and infused. MSCs were obtained from the bone marrow of haploidentical family or third party unrelated donors.

Grading of Adverse Events

Immediate AEs were monitored after each CB unit infusion every 15 minutes for the first hour, hourly for 2 hours, and then at 24 hours following the second CB infusion. Adverse events were graded on a scale of 1–5 according to the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0.

Statistical Methods

The analyses were primarily descriptive, including the median and range of clinical variables. Comparisons between multiple groups were performed using Analysis of Variance and Student's t-test for continuous variables, and Chi-squared test and Fisher's Exact Tests were performed for categorical data. A p-value <0.05 was considered statistically significant.

RESULTS

The median age of all enrolled patients was 42.8 years (range 3–74) and was similar for recipients of unmanipulated versus expanded CB units. There was a significant gender difference among the unmanipulated and MSC expanded cohorts (Fisher's Exact Test; p=0.01). Most patients received CB transplantation for the treatment of acute leukemia (AML: n=56, ALL: n=26). A smaller subset of patients was treated for chronic leukemia (CML: n=6, CLL: n=14) and lymphoma (Hodgkin: n=9, Non-Hodgkin: n=21). All four patients with myelodysplastic syndrome received cytokine-expanded CB. Both the cytokine

expanded and MSC expanded cohorts were noted to have a larger number of patients having undergone prior stem cell transplantation compared to the unmanipulated group (Fisher's Exact Test; p=0.006 and p=0.009 respectively). Similar numbers of patients received CB transplantation with ABO-mismatched units. Patients administered cytokine-expanded or MSC-expanded CB transplantation received larger fluid volumes in comparison to patients receiving two unmanipulated CB units (Student's t-test; $p < 0.001$). Furthermore, patients administered MSC-expanded CB progenitors received greater fluid volumes than cytokine-expanded CB recipients due to the additional infusion of the negative cell fraction (CD133-containing T cells and other immune cells which was cryopreserved as the CD133+ cells were expanded and then both fractions were infused together) (Student's t-test; $p < 0.001$). Importantly, there was no significant difference in the incidence of AEs among the unmanipulated, cytokine expanded, and MSC expanded cohorts (Chi-squared test; p=0.85).

Among the 274 CB unit infusions in 137 patients, there were a total of three Grade 2 and two Grade 3 infusion reactions occurring within 24 hours of CB transplantation (Table 2), resulting in an overall AE rate of 3.7% (2.2% Grade 2, 1.5% grade 3). The majority of the AEs manifested as signs of hypertension and shortness of breath and was largely responsive to diuretic administration. Most AEs occurred following infusion of the unmanipulated CB product. Two Grade 2 AEs occurred in the unexpanded cohort and manifested as asymptomatic hypertension that responded to anti-hypertensive medication. Two Grade 3 AEs were noted in recipients of CB expanded with the liquid culture system. One patient experienced loss of consciousness after infusion of the expanded CB unit that was likely the result of a vasovagal hypotensive episode. The patient required intravenous fluids and symptoms resolved within 3 hours. The second patient to experience a Grade 3 event developed hypertension and shortness of breath following infusion of the unmanipulated CB unit. The patient was administered anti-hypertensive medication and supplemental oxygen then transferred to the intensive care unit for respiratory distress. Prior to transfer the patient received the second expanded CB unit. Finally, a single Grade 2 AE was noted in recipients of MSC-expanded CB units. One patient developed shortness of breath following infusion of the expanded CB unit. The symptoms soon resolved after the administration of diuretics and supplemental oxygen.

Among the 5 patients experiencing immediate AEs, we failed to detect any association of AEs with age, gender, disease, transplant history, premedication, ABO compatibility or total infusion volume (Table 3). Importantly, there was no association between AEs and a particular treatment group.

DISCUSSION

With over 600,000 CB units available worldwide and over 20,000 CB transplants performed to date, CB transplantation is now considered a desirable option for patients lacking HLA-matched donors. Historically, the use of CB has been hindered by delayed engraftment and an increased risk of infection as a result of the low total nucleated and CD34+ cell doses in the CB units. Current strategies have attempted to overcome these limitations by either enhancing engraftment via transplantation of multiple CB units[15] and ex vivo expansion of

CB progenitors[10,11] or by developing novel methods to prevent opportunistic infection[16] and GVHD.[17]

Adverse events following CB infusion are typically mild and transient. Up to 20– 60% of patients may experience hypertension, bradycardia, chest tightness or nausea.[18,19] Rarely, patients may suffer severe complications such as cardiac ischemia, pulmonary edema, and acute renal failure.[20,21] These AEs have been speculated to be the result of acute volume expansion, conditioning regimens, transfusion-related acute lung injury, and cell lysis products.[19,20] Furthermore, serious AEs have been attributed to components of the cryopreservation solution such as DMSO and Dextran-40.[21–24]

CB expansion requires extensive manipulation and co-culture with cytokines or various cell types that could dangerously alter the CB product. Previous studies have evaluated the safety of methods aimed at increasing cell viability and decreasing the incidence of AEs, such as RBC depletion and washing the unit prior to infusion, but to our knowledge the safety of infusing ex vivo expanded CB units has not been evaluated. Here we report that the infusion of ex vivo expanded CB is safe and not associated with an increased incidence of AEs in comparison to unmanipulated CB infusion, regardless of the method of ex vivo expansion. We detected a total of 5 AEs yielding an AE rate of only 3.7%. The majority of AEs manifested as hypertension that were adequately managed with anti-hypertensive medications. There did not appear to be an association of AEs with patient age, gender, disease, prior stem cell transplant, premedication, ABO compatibility or total infusion volume; patient engraftment, GvHD, chimerism, and survival data can be found in this recent report.[11]

In conclusion, the ex vivo expansion of CB cells – whether by cytokine expansion or co-culture with MSCs – did not increase the incidence of AEs in 89 patients receiving one unmanipulated unit and one expanded unit when compared to 48 patients receiving two unmanipulated units. Our findings suggest that the infusion of unmanipulated followed by expanded CB products is a safe procedure associated with a low probability of inducing severe reactions.

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Abbreviations

AE	Adverse Event
CB	Umbilical Cord Blood
HLA	Human Leukocyte Antigen
GVHD	Graft-Versus-Host Disease
TNC	Total Neutrophil Count
RBC	Red Blood Cell

DMSO Dimethyl sulfoxide

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Table 1

Patient Characteristics

		Unmanipulated (n=48)	Cytokine Expanded (n=46)	MSC Expanded (n=43)	p-value
Age					0.32
	Mean	43.9	39.9	44.8	
	Median	43.5	40.5	45.0	
	Range	5 – 74	3 – 66	5 – 69	
Gender					0.02
	Male	22 (44.8%)	27 (58.7%)	32 (76.7%)	
	Female	26 (55.2%)	19 (41.3%)	11 (25.6%)	
Disease					0.04
	AML	20 (41.7%)	23 (50.0%)	13 (29.5%)	
	ALL	14 (29.2%)	5 (10.9%)	7 (15.9%)	
	CML	3 (6.3%)	0 (0.0%)	3 (6.8%)	
	CLL	3 (6.3%)	4 (8.7%)	7 (15.9%)	
	HL	2 (4.2%)	2 (4.3%)	5 (11.4%)	
	NHL	6 (12.5%)	7 (15.2%)	8 (18.2%)	
	MM	0 (0.0%)	1 (2.2%)	0 (0.0%)	
	MDS	0 (0.0%)	4 (8.7%)	0 (0.0%)	
Previous Transplant					0.01
	No	45 (93.7%)	33 (71.7%)	31 (72.1%)	
	Yes	3 (6.3%)	13 (28.3%)	12 (27.9%)	
Premedication					0.50
	HCT	3 (6.2%)	13 (28.3%)	12 (27.9%)	
	HCT + DPH	45 (93.8%)	33 (71.7%)	31 (72.1%)	
ABO Mismatch					0.49

		Unmanipulated (n=48)	Cytokine Expanded (n=46)	MSC Expanded (n=43)	p-value
	CB #1	28 (58.3%)	27 (58.7%)	25 (58.1%)	
	CB #2	30 (62.5%)	20 (43.5%)	22 (51.2%)	
Mean Infusion Volume (mL)		(Range)			<0.001
	CB #1	94.4 (26–155)	109.8 (45–229)	104.9 (58–249)	
	CB #2	96.0 (30–149)	122.1 (39–212)	106.5 (65–279)	
	Negative Fraction	n.a.	n.a.	58.8 (57–114)	
	Total	190.4 (60–301)	232.0 (90–352)	270.2 (217–427)	
Adverse Event					0.85
	Incidence	2 (4.2%)	2 (4.3%)	1 (2.3%)	

Abbreviations: ALL; Acute Lymphocytic Leukemia, AML; Acute Myelogenous Leukemia, CB; Cord Blood, CLL; Chronic Lymphocytic Leukemia, CML; Chronic Myelogenous Leukemia, DPH; Diphenhydramine, HCT; Hydrocortisone, HL; Hodgkin Lymphoma, NHL; Non-Hodgkin Lymphoma, MDS; Myelodysplastic Syndrome, MSC; Mesenchymal Stromal Cells

Table 2

Summary of Adverse Events

Cohort	Adverse Event	Infusion #	CB unit	Grade
Unmanipulated	HTN	1	Unmanipulated	2
	HTN	1	Unmanipulated	2
Cytokine Expanded	LOC with ICU transfer	2	Expanded	3
	HTN and Hypoxia	1	Unmanipulated	3
MSC Expanded	SOB	1	Expanded	2

Abbreviations: HTN; Hypertension, ICU; Intensive Care Unit, LOC; Loss of Consciousness, SOB; Shortness of Breath

Table 3

Association of Adverse Events

		Adverse Event		
		Yes (n=5)	**No (n=132)**	**p-value**
Age (yr)				0.28
	Mean	29.2	43.4	
	Median	19	42.5	
	Range	5 – 74	3 – 66	
Gender				0.07
	Male	5 (100%)	76 (57.6%)	
	Female	0 (0%)	56 (42.4%)	
Disease				0.76
	AML	1 (20%)	55 (41.7%)	
	ALL	2 (40%)	24 (18.2%)	
	CML	0 (0%)	6 (4.5%)	
	CLL	0 (0%)	14 (10.6%)	
	HL	1 (20%)	8 (6.1%)	
	NHL	1 (20%)	20 (15.2%)	
	Other	0 (0%)	5 (3.8%)	
Prior Transplant				1.00
	No	4 (80%)	105 (80%)	
	Yes	1 (20%)	27 (20%)	
Premedication				1.00
		0 (0%)	8 (6.1%)	
	Full	5 (100%)	124 (94.0%)	
ABO Compatibility				1.00
	No	4 (80%)	101 (76.5%)	
	Yes	1 (20%)	31 (23.5%)	
Total Infusion Volume				0.09
	Mean	175.6	231.1	
	Median	180.0	230.5	
	Range	90 – 244	60 – 427	
Cohort				0.85
	Unmanipulated	2 (40%)	46 (34.8%)	

	Adverse Event		
	Yes (n=5)	**No (n=132)**	**p-value**
Cytokine Expanded	2 (40%)	44 (33.3%)	
MSC Expanded	1 (20%)	42 (31.8%)	

Abbreviations: ALL; Acute Lymphocytic Leukemia, AML; Acute Myelogenous Leukemia, CB; Cord Blood, CLL; Chronic Lymphocytic Leukemia, CML; Chronic Myelogenous Leukemia, HL; Hodgkin Lymphoma, MSC; Mesenchymal Stromal Cell, NHL; Non-Hodgkin Lymphoma, MDS; Myelodysplastic Syndrome, MSC; Mesenchymal stromal cells