Unrelated Umbilical Cord Blood Transplant for Children with β -Thalassemia Major

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Abstract Beta thalassemia major, one of the most prevalent hemoglobinopathy throughout the world, can be cured by allogeneic stem cell transplantation (SCT) (Bone Marrow Transplant 36:971–975, 2005). Many patients, however, lack a suitably matched related sibling donor. Unrelated umbilical cord blood (UCB) can be used as an alternative stem cell source for these patients. This report describes SCT for nine children with beta-thalassemia major using partially HLA-matched unrelated UCB. Conditioning included oral busulfan 16 mg/kg (day –10 to –7), cyclophosphamide (Cy) 200 mg/kg (day –5 to –2), fludarabine 90 mg/kg (day –13 to –11), and antithymocyte globulin (rabbit) 7.5 mg/kg (day –3 to –1). The infused cell dose was 10.71×10^7 /kg total nucleated cells (TNC) (range 6.5–17 $\times 10^7$ /kg TNC). The patients ranged in age from 1.5 to 7 years, in weight from 10.5 to 17 kg. A second transplant with two unrelated cord blood units was attempted in two patients who had primary graft failure. The retransplant recipients were preconditioned with i.v Cy 120 mg/kg (day –3 to –2). Five of the nine patients engrafted promptly with 50–100 % donor chimerism (56 %). They engrafted at a median of 17 days (range 12–19). One patient is transfusion free for 36 months; a second patient is transfusion free for 18 months and a third is transfusion free for 9 months. There was no transplant related mortality. Four of the nine children had autologous

recovery without engraftment. Primary graft rejection is the major complication. Post transplant complications were mild hepatic veno-occlusive disease, acute GVHD grade II, and CMV interstitial pneumonia. The chronic GVHD was limited and could be controlled by Methylprednisolone combined with Mycophenolate. The lack of a marrow donor registry in India makes UCBT from related and unrelated donors a good alternative. Transplant should be delayed until the child is at least 18 months of age. The dose of UCB stem cells is the most important factor for engraftment. UCB has the advantages of rapid availability and low risk of severe GVHD despite donor–recipient HLA disparity (Transplant Proc 37:2667–2669, 2005). We demonstrate the feasibility of this procedure in the setting of a developing country.

Keywords Unrelated umbilical cord blood transplantation · Transfusion dependent thalassemia · Engraftment

Introduction

The thalassemia syndromes are a heterogeneous group of inherited anemias characterized by defects in the synthesis of one or more of the globin chain subunits of the haemoglobin tetramer. As a group, they represent the most common single genetic disorder known. In many parts of the world, they constitute a major public health problem.

Many different mutations cause β -thalassemia and its related disorders, such as $\delta\beta$ -thalassemia and the silent carrier state. They are inherited in a multitude of genetic combinations responsible for a heterogeneous group of clinical syndromes. β -Thalassemia major, also known as Cooley's anaemia or homozygous β -Thalassemia, is a

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Table 1 Transplant characteristic of patients received UCB for thalassemia

	1	2	3	4	5	6	7	8	9
Age (years)/sex	1.5/M	2/M	2.5/M	7/M	8/M	4/F	2/M	5/M	2/M
Weight (kg)	10.5	11.4	10.5	17	17	12.5	12.3	15	9.5
Height (cm)	89	79	85	115	111	95	78	103	78
Pretransplant s. ferritin (ng/ml)	1,424	1,134	2,582	3,200	2,844	1,630		2,094	1,164
HLA matching	6/6	6/6	5/6 and 5/6	5/6 and 4/6	5/6	6/6 2nd –4/6	5/6 and 4/6	4/6 and 4/6	5/6
TNC ($\times 10^7$ /kg)	10.5	17.2	8.9	15	8.17	6.5	17.1	17.5	13.9
Cord	S	S	D	D	S	S	D	D	S
2nd transplant	N	N	Y	N	N	Y	N	N	N

M male, F female, TNC total nucleated cells, S single D double, Y yes, N no

clinically severe disorder that results from the inheritance of two β -thalassemia alleles, one on each copy of chromosome 11.

SCT still remains the only cure currently available for patients with thalassemia [1–4]. Umbilical cord hematopoietic stem cells are increasingly used as an alternative to bone marrow; advantages include ready availability, no risk to the donor, low rate of viral contamination and low risk of GVHD. Disadvantages include low stem-cell dose for larger patients and lack of stem cells for boost infusions following the initial procedure. In multiply transfused patients, rejection of donor cells is a major clinical problem due to sensitization of transplant candidates to HLA antigens as a consequence of prior red cell transfusions. We hypothesized that two partially matched umbilical cord blood (UCB) units could facilitate engraftment without crossed immunological rejection. We discuss the newer strategies being pursued to improve the safety and efficacy of unrelated UCB transplantation (UCBT).

Patients and Methods (Table 1)

Nine patients with transfusion-dependent thalassemia underwent transplantation with one or two unrelated UCB units at the Gujarat Cancer and Research Institute (GCRI) between March 2008 and July 2011. The thalassemia patients were diagnosed before or around 1 year of age and thereafter received regular monthly red cell transfusion. All but one of our patients received more than 100 U. No patient was splenectomized before transplantation. The diagnosis of each transfusion-dependent thalassemia participant was confirmed by DNA analysis. Patients included in this study did not have donor relatives with acceptable HLA compatibility. After written informed consent given by their caregivers, nine children were eligible for unrelated UCBT.

We did not assess risk in our patients according to the Pesaro risk classification because we did not routinely perform pretransplantation liver biopsy. The median age of

the recipients was 2.5 years (range 1.5–8 years) and median weight was 12.3 kg (range 9.5–17 kg).

Cord Blood Selection and Characteristics

Units were considered initially if their HLA-A, -B, and -DR phenotypes matched at least four of the patient's submitted six antigen HLA phenotypes at a serologic or intermediate-DNA level of resolution. The compatibility of HLA-A, -B, and -DRB1 was further confirmed by high-resolution PCR technique with sequence-specific primers (PCR-SSP). HLA disparity between each unit and the recipient and between two units did not necessarily have to be at the same loci. Searches for unrelated cord blood donors were processed through the StemCyte Cord Blood Bank, Taiwan and Reliance.

Conditioning Regimen and Transplantation Procedure

Before the UCB transplant procedure, all patients were placed in a high-efficiency particulate air-filtered room in the bone marrow transplantation unit. The preparative regimens consisted of oral busulfan 16 mg/kg (day –10 to –7), cyclophosphamide 200 mg/kg (day –5 to –2), fludarabine 90 mg/kg (day –13 to –11), and antithymocyte globulin (rabbit) 7.5 mg/kg (day –3 to –1). During treatment, patients received valproate (10 mg/kg—1st dose then 4–5 mg/kg 8 h) for prophylaxis against seizures. Mesna (50 mg/kg) was administered intravenously on the days of cyclophosphamide infusion. GVHD prophylaxis comprised cyclosporine (CsA) (2.5 mg/kg intravenously every 8 h) from day –3 with a course of methylprednisolone (1 mg/kg intravenously every 12 h on days 5–19, decreasing 25 % thereafter every other day). The level of CsA was adjusted to between 200 and 400 ng/ml, as measured by radioimmunoassay. The CsA dose was tapered beginning at least 60 days after demonstrating engraftment and full donor chimerism by variable number tandem repeat (VnTR) analysis.

In this study, four patients received 2 U of cord blood while others received a single cord blood unit. Cord blood source was ‘Reliance life science’ in six patients and ‘Stemcyte’ for three patients. In two patients we attempted unsuccessful second unrelated UCBT after failure of first. In double cord transplant, the DNA of only one of the two donors was eventually detectable in patients with durable engraftment. The numbers of infused total nucleated cells are shown in Table 1. The UCB units were thawed with gentle agitation in a 37 °C water bath and without further processing before infusing into the patients. Filgrastim 10 g/kg/day was given subcutaneous on day 1 after transplantation and on each day thereafter, until the neutrophil count remained above $1.0 \times 10^9/l$ for 3 consecutive days.

Supportive Care and Post Transplantation Follow-up

Blood components were given whenever indicated to maintain haemoglobin and platelet values >80 g/l and $20 \times 10^9/l$ respectively. For fungal infection oral itraconazole (antifungal prophylaxis) 3 mg/kg daily was prescribed for the month preceding transplantation. Oral acyclovir and oral co-trimoxazole were given to prevent CMV reactivation and *Pneumocystis jiroveci* infection. Both acyclovir and co-trimoxazole were continued as prophylaxis until day 180 after transplantation and further continued until T cell function is restored. Parenteral nutrition was provided for the duration of anorexia in form of 25 % dextrose, amino acid and albumin. Intravenous immunoglobulin (500 mg/kg) was given at days –6, +7, +21, +35, +56, +77, and +98 following UCBT. The standard CMV pp65 antigenemia assay was performed in parallel. The positive results were quantified by counting the number of pp65-expressing cells per 50,000 leukocytes on the slide.

Myeloid engraftment was defined as three consecutive days of an absolute neutrophil count of $0.5 \times 10^9/l$. The last day of RBC transfusion was recorded as a day of RBC transfusion independence. Platelet engraftment was defined as 7 consecutive days of a platelet count of $20 \times 10^9/l$ maintained without transfusion. Current methods for measuring hematopoietic chimerism are based on STR polymorphisms that distinguish recipient from donor. Serial STR polymerase chain reaction confirmed the conversion from mixed chimerism to a predominantly donor profile on the day the myeloid engraftment occurred, on days +30, +60, +180, +270 and +360, 1.5 years after transplantation, and yearly, thereafter. An additional test was done if clinically indicated. In patients achieving myeloid engraftment, desferrioxamine was administered at a dose of 40 mg/kg/day as a 24-h intravenous infusion to accelerate the clearance of body iron deposits.

Results

Engraftment and Chimerism Status

Five of the nine patients engrafted promptly with 50–100 % donor chimerism (56 %). Two children had 100 % donor chimerism on day +30 which is persistent till last follow-up. Three children had 50 % donor chimerism on day +30, 1 of these has 63 % donor chimerism on day +60 and the other two lost engraftment promptly (autologous recovery on day +60). Four children had autologous recovery without engraftment.

The absolute neutrophil count first exceeded $0.5 \times 10^9/l$ on median day 17 (range 14–25 days), and the patient became red cell- and platelet-independent on days 32 and 36, respectively. All patients engrafted at a median of 17 days (range 12–19). First patient is transfusion free for 40 months, second patient is for 21 months and 3rd patient is from 7 months. There is no transplant related mortality (TRM). Primary graft rejection is major complication and was seen in 4 of 9 patients.

The post transplant complications were mild hepatic veno-occlusive disease, acute GVHD grade II, and CMV interstitial pneumonia. The chronic GVHD was limited and could be controlled by Methylprednisolone combined with Mycophenolate.

Double cord blood units were used to minimize the risk of developing graft failure in these multiply transfused patients with weights over 30 kg.

Transplantation-Related Events

The grading of acute GVHD was grade I in one patient and grade II in two. Limited chronic GVHD involving skin subsequently developed in one patient who was treated with topical steroids. One patient developed CMV pneumonitis treated successfully with ganciclovir. Asymptomatic CMV reactivation was detected in seven patients and all were successfully treated pre-emptively with intravenous ganciclovir. One patient developed late-onset hemorrhagic cystitis 22 days post transplant associated with BK viruria concomitant with CMV reactivation. BK virus-associated hemorrhagic cystitis and simultaneous CMV reactivation were treated simply by stopping immunosuppressive agents after complete autologous recovery had been identified.

One patient developed gram negative septicemia and treated with antibiotics according to culture and sensitivity.

Discussion

We describe the results of our UCB SCT programme in the setting of a developing country and demonstrate the

Table 2 Characteristics of engraftment, transplant related events, GVHD grades, outcome and chimerism

	1	2	3	4	5	6	7	8	9
ANC $>0.5 \times 10^9$	14	21	20	14	21	19 2nd –22	25	14	17
Transfusion independence	18	32					35		
Platelet count $> 20,000$	38	28	28	49	39	29 2nd –32	60	20	36
Transplant related complication	CMV Pneumonitis	CMV+	Gram negative septicemia CMV+ Hemorrhagic cystitis	CMV+	CMV+	CMV+	CMV+		CMV+
Acute GVHD	Skin grade II Git grade II						Skin grade II Git grade II		
Chronic GVH		II/SKIN							
Outcome	TI	TI	AR	AR	AR	AR	TI	AR	AR
Month of transplant	10–09	3–08	5–08	8–09	9–09	12–09 and 2–10	1–11	6–11	7–11
Chimerism status (%)									
Day-30	100	100	00	00	50	0	50	50	00
Day-60	100	100	00	00	00	0	63	00	00
Latest follow-up	TI from last 21 months	TI from last 40 months	AR	AR	AR	AR	TI from last 7 months	AR	AR

TI transfusion independence, AR autologous recovery

feasibility of this approach. UCB has the advantages of rapid availability, and low risk of severe GVHD despite donor–recipient HLA disparity [5]. The low mortality risk outweighs the benefit from unrelated transplantation and cell dose is a critical factor for UCBT [6], although it is difficult to compare the units from different cord blood banks. Laughlin et al. [7] found that dose of cryopreserved nucleated cells is a major determinant of neutrophil recovery and that higher CD34⁺ cell dose is associated with improved survival in adult UCB recipients. The optimal way to deal with delayed engraftment of UCB and its complications has not yet been found [8].

Although the use of myeloablative conditioning might be linked to infertility, its benefits should be weighed against its potential risks, and diligent long-term follow-up should be carried out especially in thalassemia patients with iron overload, in whom the risk for infertility is already increased. After transplantation of cord blood, engraftment usually seems slower than after transplantation of marrow or peripheral blood. A consensus is emerging that UCB grafts of higher cell doses should be selected wherever possible to optimize engraftment [9]. Although CD34 quantification in UCBT has not been consistently predictive of time to donor hematopoietic engraftment [10], CD34 cell dose may play a critical role in engraftment. Thus, there are concerns about reaching a sufficient cell dose for engraftment.

UCBT has problems related to risk of graft failure or relatively late engraftment in patients with thalassemia [11, 12].

Patients with transfusion-dependent thalassemia are at high risk of graft failure, either because of major prior alloimmunization or an insufficient amount of UCB stem cells [13]. Our conditioning regimens were generally well tolerated and the median day of neutrophil engraftment was 17 days (14–25 days) after transplantation. Three patients had sustained engraftment while two cases were subsequently lost engraftment (33 %). Although agreement on the minimum acceptable and the optimal UCB graft cell doses are not yet unanimous, [14] our result suggested that higher cell doses may partially overcome the negative impact of HLA disparity. Ideally, since no standardized treatment concept exists, these data will be used to design larger comparative studies.

UCB transplantation from related and unrelated donors should be considered for patients with beta-thalassemia major who lack traditional bone marrow donors. Newborns should undergo screening for hemoglobinopathy, so disease could be transplanted early in life before experiencing the morbidity and mortality caused by transfusion therapy, alloimmunization, and iron overload, increasing the likelihood of successful transplantation therapy.

As there is no marrow donor registry in India, UCB transplantation from related and unrelated donors is a good

alternative and is feasible. It should be considered for patients with beta-thalassemia major who lack traditional bone marrow donors. Transplant should be done after 18 months of age at the earliest. Dose of stem cells is the most important factor for engraftment. UCB has the advantages of rapid availability and low risk of severe GVHD despite donor–recipient HLA disparity [2] (Table 2).

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