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Case Report

Dimethyl sulfoxide toxicity in umbilical cord blood transplantation in patients less than 4.5 kilos of weigh

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Introduction

In primary immunodeficiencies, including severe combined immunodeficiency (SCID), the only potentially curative therapy is allogeneic hematopoietic stem cell transplantation (HSCT), which should be performed as soon as possible. There are several sources of hematopoietic stem cells, including bone marrow, peripheral blood stem cells (PBSC) and umbilical cord blood.

On the other hand, dimethyl sulfoxide (DMSO) is the most used cryoprotectant for hematopoietic progenitors cell storage; nevertheless this molecule is related to several adverse events (AEs) at the time of its infusion. Although there is no cutoff point in patient weight that determines an increased risk of complications secondary to the toxicity of DMSO, there is a relationship between a lower volume of DMSO per kilo of weight infused with a higher probability of an uneventful stem cell infusion.

We report three successful cases of umbilical cord blood transplantation (UCBT) after a reduce intensity conditioning (RIC) regimen in infants with 4.5 kilos of weight or less with SCID, without side effects due to DMSO. In these three patients, the risk of toxicity was important because of their low weight.

Case report

Case 1

A two-month old, 2.4 kg Hispanic female (Table 1) with SCID was treated with intravenous gamma globulin once per week. She was transplanted with a 4/6 mismatch Hispanic cord blood unit (CBU) using a RIC regimen consisting of fludarabine 40 mg/m^2/day for three days, cyclophosphamide 350 mg/m^2/day for three days and a single dose of melphalan 140 mg/m^2.

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Table 1 – Demographic data.

Case no.	Age (months)	Sex	Weight (kg)	Total infused volume (ml)	Volume infused ml per kg	Complications	Outcome
1	2	F	2.4	17	7	None	Alive
2	4	F	3.7	47	12.7	Irritability	Alive
3	9	M	4.3	18	4.18	None	Alive

Table 2 – Total nucleated cells (TNC) data.

Case no.	TNC Before freezing	TNC Post-thaw	TNC Post-thaw Recovery
1	35.48×10^7/kg	29.1×10^7/kg	82%
2	26.85×10^7/kg	21.48×10^7/kg	80%
3	24.7×10^7/kg	20.3×10^7/kg	82%

Cyclosporine 5 mg/kg/4 days was administered for GVHD prophylaxis. Intravenous clorphenamine 0.2 mg/kg, ondansetron 0.2 mg/kg, and hyperhydration were used to prevent DMSO toxicity. The CBU was infused immediately after thawing and washing DMSO with Rubinstein's method without any modification of the process, with no side effects during infusion. She received 29.1×10^7/kg of total nucleated cells (TNC) (Table 2) and 8.6×10^5/kg of CD34+ cells (Table 3). She engrafted $>0.5 \times 10^9$/L neutrophils on day +15, and $>20 \times 10^9$/L platelets on day +19. Forty-one months after transplantation, full engraftment was achieved, and the patient was free of infections and medications.

Case 2

A 4-month old Hispanic 3.7 kg female (Table 1) with SCID was treated with intravenous gamma globulin once per week. She received a 5/6 HLA mismatch CBU, with the same RIC regimen and GVHD prophylaxis as described above. The premedication used to prevent DMSO toxicity was the same as that in case one. CBU was infused after thawing and washing. The patient presented irritability as a side effect during the infusion. She received 21.48×10^7/kg of TNC (Table 2) and 12.1×10^5/kg of CD34+ cells (Table 3). She engrafted $>0.5 \times 10^9$/L neutrophils on day +13, and $>20 \times 10^9$/L platelets on day +15. Thirty-five months after transplantation the patient was fully engrafted and free of infections and medications.

Case 3

A 9-month old Hispanic 4.3 kg male (Table 1) diagnosed with SCID treated the same as Cases 1 and 2. He was transplanted with a 5/6 HLA mismatch CBU, using the same RIC regimen, GVHD and DMSO toxicity prophylaxis, as in the other two patients. CBU was infused after washing with no side effects. He received 20.3×10^7/kg of TNC (Table 2) and 1.7×10^5/kg of CD34+ cells (Table 3). He engrafted $>0.5 \times 10^9$/L neutrophils on day +11, and $>20 \times 10^9$/L platelets on day +17. Thirty-two months after transplantation the patient is 95% engrafted and free of infections and medications.

In the cases presented above, vital signs were monitored during hematopoietic cell infusion, without significant changes, except for heart rate from 120 to 154 beats per minute in case number 2, which was related to irritability of the patient and was considered within normal limits. Chimerism analyzed on day +30 after transplantation in each patient was 95%, 100%, and 90%, respectively.

Discussion

To preserve hematopoietic progenitor units, it is necessary to freeze them in liquid nitrogen at storage temperatures ranging from −196° to −150 °Celsius.[1] For long-term preservation, the use of substances such as DMSO is helpful for cryoprotection and preservation of cell viability.

DMSO is a small amphipathic molecule with a highly polar domain and two apolar groups, making it soluble in aqueous and organic media. Due to its vast pharmacokinetic volume distribution affects multiple organ systems with large spectrum toxicities, including blood- brain barrier penetration.[1] FDA has approved DMSO since 1978 for intravesical instillation and for treating musculoskeletal, dermatological, urinary, pulmonary, renal diseases and even cerebral edema. This cryopreservative additive (CPA) is used as the standard method, at a 10% concentration, for HSCT in most institutions.[1–10]

DMSO AEs occur in both, children and adults, although they are expected to be greater in children due to the amount of DMSO per kg of weight. Toxicity is related to the amount of DMSO in the HSCT cryopreserved units and the maximum dose allowed is 1 g/kg of recipient body weight. DMSO can cause diverse systemic AEs such as nausea, vomiting, rashes, massive cardiac arrest, renal failure and neurological complications.[2] These effects may be derived from DMSO-induced histamine release, others could be directly related to the

Table 3 – CD34+ cells data.

Case no.	CD34+ Before freezing	CD34+ Post-thaw	Post-thaw Recovery cells	Post-thaw viability (by flow cytometry)
1	13.7×10^5/kg	8.6×10^5/kg	62.77%	87%
2	12.21×10^5/kg	12.1×10^5/kg	99%	88.6%
3	2.68×10^5/kg	1.7×10^5/kg	63.43%	97%

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amount of DMSO infused and several of the cardiovascular side effects could be multifactorial.[1–4] Ikeda et al. reported that after infusion of umbilical cord blood units, the most frequent AE is bradycardia, while in cryopreserved bone marrow units was hypertension.[4,5] Central nervous AEs that may be associated with DMSO are epileptic seizures, stroke, transient and temporary leukoencephalopathy and global amnesia.[6] It is worth noting that AEs are generated by DMSO, but also possibly by alloantigens or a large volume per recipient body weight.[5]

A study by Y. Okamoto et al. assessed the toxicity observed after transfusion of cryopreserved and thawed-without washing PBSC in children aged 1 to 16 years. The patients' body weights ranged from 9 to 78 kg. They reported mild AEs in most pediatric patients after thawing cells from DMSO. The most common toxicities were hemoglobinuria (74.1%), headache (70.4%), nausea (68.5%) and vomiting (46.3%). Only 15% of the patients had more severe symptoms such as transient shock; however, patients recovered promptly and completely.[7] Severe toxicity tends to appear mostly in non-washed infusions, leaving the washing protocol as essential element for a successful hematopoietic transplant without untoward reactions, as in the cases described by Sanchez-Salinas.[2]

Although thousands of stem cell units from different sources are cryopreserved using DMSO and transfused each year, there have been few reports on the incidence of AEs in children weighting less than 5 kg with cryopreserved products. While Ikeda et al. mentioned gastrointestinal manifestations, bradycardia, hypertension and allergic reactions in the pediatric population, in our three cases, there were no AEs except for irritability.[3–5] We can only assume that this patient had abdominal or retrosternal pain, or even nausea, and this was the reason for the irritability, even though the 3 patients were premedicated with an antihistamine and ondansetron, in order to avoid them. However, in our experience (data not published), in 146 cryopreserved units infused in children and adults, nausea, vomiting, bradycardia, hypotension and desaturation were presented in an adult patient, so that even adults should be monitored during the infusion of cryopreserved hematopoietic progenitor cells.

Syme et al. compared a non-washed-cells auto graft group versus a washed-cells auto graft group[8] and found AEs in both groups. Some symptoms, most of them gastrointestinal, were more common in the non-washed-cells group. Nevertheless, symptoms such as mucositis, fatigue, facial flushing and discomfort were similar in both groups. They reported as well one patient who experienced a grade 1 cardiac AE in the washed-cell group. This may help to prove that washing the cells does not exempt patients from presenting AEs.

There are currently some alternatives to reduce patient exposure to DMSO by a prolonged infusion, DMSO depletion after thaw-processing, or using alternative CPAs with or without DMSO.[1]

The current standard post-thaw depletion method, or Rubinstein's method[9], includes a wash step to remove DMSO, lysed red cells, and remove stroma. The wash step procedure was performed in our laboratory with 20% human albumin (Grifols Biological, Inc. Los Angeles, USA) mixed with Saline 0.9% (Laboratorios PiSA, Mexico City, Mexico) to create a 5% human albumin solution and Dextran 40 (Rheomacrodex®, Laboratorios PiSA, Mexico City, Mexico). Most institutions included this method in patients under 20 kg of weight due to the risk of reaching the recommended maximum dose of DMSO. Dymethil sulfoxide-depletion has shown a potential benefit in reducing the risk of events due to DMSO toxicity; however, when DMSO wash process is made, it might be responsible for nearly half of the cell loss of the infused unit. To make the best decision, these two scenarios should be weighed.[10] Worth noting, when DMSO is washed, it does not completely prevent the occurrence of AEs related to the infusion. This process only minimizes the incidence of AEs, as anaphylaxis is not dose dependent and in Rubinstein's method there is a residual amount of DMSO that remains in the unit to be infused.

On the other hand, engraftment after UCBT is highly dependent on the TNC dose per kg of weight. In low weight patients, DMSO toxicity can be reduced, without cellular death being a problem, because it is easy to find CBUs to transplant these small patients. However, we cannot forget that it is also true that the lower the weight of the patient, the greater the residual amount of DMSO per kilo of weight, which may lead to AEs in low-weight patients.

To our knowledge, there are few reports of DMSO adverse events in children weighting less than 5 kg, possibly because UCBT in pediatric patients is usually performed with CBU washed by Rubinstein's method. This method seems to be the most convenient technique to process hematopoietic progenitor units, guaranteeing TNC recovery and preserving cell viability[9], at least in pediatric patients.

The patients presented in this report have in common that the CBUs were washed after thawing. In our experience of 65 non-washed after thawing placental blood allografts (data not published), only three cases presented DMSO toxicity (3/66=4.54%). The side effects were acute renal failure, anaphylaxis, hemolysis and arrhythmia, all of which resolved. This supports the hypothesis of Sánchez-Salinas[2] regarding the benefits of washing cells after thawing. Further studies are required to confirm these findings.

Conclusion

DMSO toxicity in UCBT in pediatric patients may be a serious complication that must be monitored. Using low doses of DMSO obtained by washing CBUs may be safer than using non-washing units, with a minimum and transient risk of toxicity. This could be the best way to transplant children under 10 kg of body weight. Although washing the CBU can cause cell loss, in these children, the loss is unlikely to have an impact at the time of transplantation, since the cell ratio per kilogram of weight remains high after washing. However, there is always a risk of serious allergic reactions, even with a minimum amount of DMSO.

Conflicts of interest

The author declares no conflicts of interest.

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