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IMPLEMENTING CRYOPRESERVATION OF HEMATOPOIETIC PROGENITORS TO IMPROVE PRODUCT QUALITY AND INFUSION TOLERANCE

Hematology

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ABSTRACT

Introduction: Dimethyl sulfoxide (DMSO) is the most frequently used cryoprotective agent for freezing hematopoietic progenitors for transplantation. DMSO can cause adverse effects during cellular product infusion. In this setting, we reduced the DMSO concentration from 10% to 5% as a strategy to reduce toxicity.

Methods: We retrospectively analyzed the characteristics of hematopoietic progenitor products and infusion tolerance in 240 adult patients who underwent autologous peripheral blood transplantation between January 2008 and February 2019. Patients were divided into two groups according to DMSO concentrations, 10% versus 5%.

Results Post-thawing cell viability was significantly higher in the 5% DMSO group (69.15% vs. 63.85%; p=0.028). In both groups, no statistically significant differences were observed in the total content of CD34+×106/kg progenitor cells infused (DMSO 5% 2.98 vs. DMSO 10% 3.20, p=0.409), or graft recovery and transfusion needs.

In all patients, the overall rate of adverse reactions was 7.5% (n=18); the rate was 4.6% (n=5) in the 5% DMSO group compared with 9.8% (n=13) in 10% DMSO (p=0.127). Severe adverse events were observed in the 10% DMSO group.

Conclusion: Reducing the DMSO concentration from 10% to 5% is safe for the patient and product, improving viability and the absence of severe adverse infusion reactions.

KEYWORDS

Cryopreservation, DMSO, Viability, Progenitors.

INTRODUCTION

Autologous transplantation is a therapeutic approach employed in several hematological neoplastic diseases. In these settings, hematopoietic progenitor cells collected by apheresis from peripheral blood are the most frequent source of stem cells[1]. Some centers have reported promising results with non-cryopreserved autologous hematopoietic stem cell transplantation[2], but the freezing of progenitors remains the most widely used option, which ensures cell survival for months or years. Accordingly, a cryoprotective solution must be added to cellular products. Dimethyl sulfoxide (DMSO) is the most frequently used cryoprotective agent in this procedure. DMSO penetrates the cell through the membrane and prevents ice crystal formation, interfering with hydrogen bond formation between water molecules[3]. At room temperature, DMSO is toxic and can cause adverse effects during product infusion, including serious cardiac and neurological toxicities[4-6].

Furthermore, other factors can influence the development of adverse effects during infusion, mainly the number of damaged granulocytes that do not survive cryopreservation[7,8]. Several centers have sought strategies to minimize the toxicity of the cryoprotectant[9,10], limiting the total amount of DMSO infused at one time. Additionally, washing protocols after thawing were used to eliminate DMSO. Although this technique reduces side effects during infusion, it can damage cells and lose progenitors due to mechanical and osmotic stress[11]. However, reports have shown that washing progenitors after thawing could delay platelet engraftment[12]. Another strategy is to decrease the DMSO concentration used during freezing. Classically, most institutions have employed 10% DMSO, although several studies have reported good outcomes with reduced concentrations, presenting lower rates of side effects[13-15].

Based on these reports, we modified our cryopreservation strategy in 2014 to improve the transplantation procedure.

The aim of this study was to evaluate the efficacy and safety of reducing the DMSO concentration from 10% to 5% for freezing peripheral blood hematopoietic progenitors for autologous transplantation at our center. We analyzed the impact on post-freezing cell viability and hemoperipheral recovery, as well as the number and severity of adverse reactions observed during infusion.

MATERIAL AND METHODS

Study Population

We retrospectively analyzed 240 adult patients older than 18 years with hematologic malignancies and who underwent autologous transplantation using peripheral blood hematopoietic progenitors at our center from January 2008 to February 2019. The following baseline characteristics were considered: sex, age, underlying disease, and conditioning regimen. The study population was divided into two groups based on the time of cell cryopreservation, as well as the DMSO concentration. Before September 2014, 10% DMSO was utilized; from October 2014, 5% DMSO was used. Only one patient in the 10% DMSO group died on day +5 before engraftment owing to septic shock and was not included in the analysis.

Mobilization And Collection

Our standard mobilization regimen involved the subcutaneous administration of 5 µg/kg/12 h granulocyte colony-stimulating factor (G-CSF; Neupogen. Amgen Europe BV. Minervum 7061, 4817 ZK, Breda, Netherlands) for 4 days, with CD34+ cell counts monitored in peripheral blood on day +5. In all cases, the minimum cut-off required to perform the apheresis procedure was 10 CD34+ cells/µL cells, determined by flow cytometry[16]. In the case of mobilization failure on the 4th day of G-CSF administration, the administration of plerixafor was considered at a standard dose of 0.24 mg/kg/day[17].

In patients who were mobilized with G-CSF associated with chemotherapy, the most commonly used regimens were ESHAP (etoposide, methylprednisolone, high-dose cytarabine, and cisplatin), with DHAP (dexamethasone, high-dose cytarabine, and cisplatin) employed in patients with lymphoma, and cyclophosphamide in multiple myeloma.

Apheresis procedures were performed using the Cobe Spectra system [COBE] (Terumo, BCT Lakewood CO) from January 2008 to May 2015 and Spectra Optia (OPTIA) (Terumo, BCT Lakewood CO) from June 2015 onward. In all cases, the program used was MNC with Cobe Spectra and cMNC with Spectra Optia, according to the manufacturer's technical specifications[18,19]. The target level of CD34+ cells in the product was > 2×10^6 CD34+/ kg for autologous transplantation.

Cryopreservation, storage, and thawing

The leukocytapheresis product was stored at 4ºC and cryopreservation was performed within the first 24 h after collection. We assessed the hematocrit, total leukocyte count, the number of CD34+ cells and viability in the fresh apheresis product and after thawing.

The final volume in each freezing bag (CryoMACS Freezing bag. Miltenyi Biotec GmbH. Friedrich-Ebert-Strabe, 68. 51429. Bergisch Gladbach, Germany) of ethylene-vinyl acetate (EVA) was between and 80-190 mL. The buffy coat was distributed in equal volumes between a minimum of 40 mL and a maximum of 95 mL in freezing bags. The cryoprotectant used was DMSO (Cryoserv, Mylan Institutional, Galway, Ireland) and the cryoprotective solution was prepared as 20% DMSO + 80% autologous plasma before September 2014, and as 10% DMSO + 90% autologous plasma after October 2014, so that the final concentrations of DMSO were 10% and 5%, respectively. The final cellular concentration in each bag was <200 $\times 10^9$/L.

The cryoprotective solution was slowly added to the cellular product in a 1:1 volume ratio to minimize cell damage. Mixing was performed on an ice plate to avoid DMSO toxicity at room temperature. Subsequently, samples were obtained for blood cultures for microbiological controls in two cryovials.

After sealing the bags, they were placed along with the cryovials in the biological freezer CM-2000 (S.E. Carburos Metálicos S.A. Madrid. Spain. Software CBIOE). This device allows the programmed freezing of bags, with controlled-rate temperature decrease through the instillation of liquid nitrogen, obtaining a freezing curve that offers a record of thermal decrease during the process. When the freezing curve reached -150ºC, freezing was completed, and all products were stored in liquid nitrogen at -196ºC.

Before infusion, the product was thawed by immersion in a liquid bath at 37º-40ºC in an overwrap bag, separating the progenitor bag from the water. In all cases, samples were obtained for bacterial cultures. The infusion was performed over a maximum period of 15-20 min immediately after thawing.

Conditioning Regimen And Transplantation Procedure

The conditioning protocol was individualized in each case based on patient characteristics and underlying disease. The most commonly used regimens were BEAM (BCNU 300 mg/m^2 intravenous (IV) on day −6, cytarabine 200 mg/m^2 IV, etoposide 150 mg/m^2 IV from day −6 to −3, and melphalan 140 mg/m^2 IV on day -2) in Hodgkin lymphoma and non-Hodgkin lymphomas, and melphalan 200 mg/m^2 or melphalan 140/m^2 in multiple myeloma.

The infusion of products was always performed by the cryopreservation laboratory staff, immediately after thawing, through a central venous catheter, by aspiration of the product from the bag and slow infusion using a standard filter for macroaggregates. The maximum administration time was approximately 15-20 min per bag. In general, no more than 10 mL/kg/day of progenitors were infused to avoid DMSO toxicity, not exceeding 1 g of DMSO per kg of receptor recipient weight and day. Patients with larger volumes received the progenitor infusion over more than one day, on consecutive days.

Thirty minutes before infusion, all patients were intravenously administered 5 mg dexchlorpheniramine, 100 mg hydrocortisone, and 10 mg metoclopramide, or 8 mg ondansetron. Vital signs (blood pressure, heart rate, temperature, and oxygen saturation) were monitored before, during, and after infusion, along with any symptom development (bradycardia, dyspnea, throat discomfort, bronchospasm, hypotension, cyanosis, fever, and chills). The severity of adverse reactions was assessed using the Common Terminology Criteria for Adverse Events Version 4.0[20].

Laboratory methods

In all cases, a daily blood count analysis was performed during the hospitalization period to evaluate the graft status, as well as the need for transfusions. CD34+ cell counts and cellular viability (percentage of alive leukocytes) were performed using 7-aminoactinomycin D (7-AAD) by flow cytometry (BD FACSCanto II).

Engraftment and supportive treatment

After hematopoietic progenitor cell infusion, leukocyte engraftment was defined for the first of three consecutive days with neutrophils > 500 cells/μL in peripheral blood, and for platelet engraftment, the first day with > 20 000 platelets/μL without transfusion. To reduce the risk of graft syndrome, no G-CSF was systematically used, with no other hematopoietic growth factors employed during the period of post-transplant aplasia, according to our center protocol.

In general, red blood cell concentrates were transfused to maintain hemoglobin values > 8 g/dL, with platelet pools transfused to maintain counts above 10 000 cells/μL, or 20 000 cells/μL if overwhelming factors were present (fever, infection, bleeding). The patient who died early post-transplant before reaching engraftment was not included in the analysis.

Statistical Analysis

Statistical analysis was performed using IBM-SPSS-25 (IBM SPSS Statistics v 25.0 for Windows; Armonk. NY, USA). All variables were analyzed to determine whether or not they adjusted to statistical normality, using histograms, normal Q-Q, asymmetry and kurtosis indices, and the Kolmogorov-Smirnov (KS) test of goodness of fit. Depending on the previous examination comparing variables with normal distribution or with a tendency toward normality with slight deviations, a test for comparison between parametric groups (Student's t-test) was employed. For asymmetric variables, the Mann–Whitney U test was used as a non-parametric alternative. The Chi-square test was used for comparing categorical variables. In all cases, the magnitude of differences was estimated by the size of the effect with Cohen's d converted to R2 equivalent[21,22]. Statistical significance was defined as a value of $p < 0.05$, except in the KS test of goodness of fit, where only serious deviations, of at least 1% ($p < 0.01$), were considered significant.

Ethical considerations

This study was made respecting the patients' health as our first consideration and all data contained in the manuscript do not allow to identify individual patients.

RESULTS

Patient Characteristics

The study population (n= 240) was separated into two groups based on the DMSO concentration used to freeze hematopoietic progenitors: 5% DMSO (n = 108) and 10% DMSO (n = 132). Baseline patient characteristics are shown in Table 1. In the 5% DMSO group, 47.2% of patients were male and 52.8% female, whereas in the 10% DMSO group, a slight female predominance of 56.1% was observed, with 43.9% of male patients. The median age at the time of transplant was 58 years in the 5% DMSO group and 57 years in the 10% DMSO. Regarding underlying diseases, multiple myeloma was the most prevalent disease in both groups (61.1% *vs.* 51.5%), followed by non-Hodgkin lymphomas (19.4% *vs.* 31.8%). No statistically significant differences were observed in any of these variables between groups. The most commonly used conditioning regimen was melphalan-200 in both groups (38.9% *vs.* 42.4%), followed by BEAM (28.7% *vs.* 37.1%). Melphalan-140 was more frequently used in the 5% DMSO group than in the 10% DMSO group (22.2% *vs.* 8.3%). These differences could be a possible limitation of the study regarding side effects.

Table 1. Patient's baseline characteristics

VARIABLES Median (range) or % (frequency)	Total (N=240)	Groups		Contrast test	
		DMSO 5% (n=108)	DMSO 10% (n=132)	value	*p*
· *GENDER* - MALE	52.1 % (125)	47.2 % (51)	56.1 % (74)	Chi^2= 1.86	.173
- FEMALE	47.9 % (115)	52.8 % (57)	43.9 % (58)		
· *AGE AT TRANSPLANTATION*	58.0 (20 – 70)	58.0 (21 – 70)	57.0 (20 – 70)	Z_U= 1.06	.288
·*UNDERLYING DISEASE* - MM	55.8 % (134)	61.1 % (66)	51.5 % (68)	Chi^2= 5.88	.118
-NHL	26.3 % (63)	19.4 % (21)	31.8 % (42)		

-HL	9.6 % (23)	12.0 % (13)	7.6 % (10)		
-Others	8.3 % (20)	7.4 % (8)	9.1 % (12)		
·*CONDITIONING REGIMEN* - BEAM	33.3 % (80)	28.7 % (31)	37.1 % (49)	Chi^2= 9.50	.023
-MEL-140	14.6 % (35)	22.2 % (24)	8.3 % (11)		
- MEL-200	40.8 % (98)	38.9 % (42)	42.4 % (56)		
- Others	11.3 % (27)	10.2 % (11)	12.1 % (16)		

Table 1. MM: multiple myeloma, NHL: non-Hodgkin lymphoma; HL: Hodgkin lymphoma; BEAM: BCNU, etoposide, cytarabine, melphalan; MEL-140: melphalan 140, MEL-200: melphalan 200. DMSO: Dimethyl sulfoxide.

Product characteristics

The product variables analyzed are shown in Table 2. We considered the total volume of the product (measured in mL), percentage of post-thawing viability (measured by flow cytometry), and the amount of CD34+×10^6 cells/kg weight of receptor recipient present in the product.

The volume of the infused product was significantly lower in the 5% DMSO group than in the 10% DMSO group (435 mL *vs*. 507 mL, p = 0.019). No statistically significant differences were observed in the total content of CD34+x10^6/kg progenitor cells infused in either group (DMSO 5% 2.98 *vs*. DMSO 10% 3.20, p = 0.409). The post-thawing cell viability was significantly higher in the 5% DMSO group (69.15% *vs.* 63.85%; p = 0.028), indicating reduced damage to leukocytes during freezing on employing a lower concentration of the cryoprotectant, consistent with the previous reports[15,23,24].

Table 2. Product characteristics, need of blood transfusions and graft recovery

	Median and range		Contrast test	
	DMSO 5% (n=108)	**DMSO 10% (n=132)**	**value**	**P**
VOLUME (ml)	435.00 (110-2 240)	507.00 (70-2 880)	Z_U=2.35 *	.019
VIABILITY (%)	69.15 (19.9-98.2)	63.85 (19.2-99.1)	t=2.21 *	.028
CD34+ x 10^6/Kg	2.98 (2-10)	3.20 (2-15.6)	Z_U=0.83 ^{NS}	.409
RBC TRANSFUSIONS	4.00 (0-16)	3.00 (0-36)	Z_U=0.19 ^{NS}	.850
PLT TRANSFUSIONS	2.50 (0-26)	3.00 (0-77)	Z_U=0.77 ^{NS}	.441
ANC>500 cells/µL	14.00 (9-26)	15.00 (10-64)	Z_U=0.93 ^{NS}	.355
ANC>1000 cells/µL	16.00 (10-27)	16.00 (10-67)	Z_U=0.93 ^{NS}	.355
PLT RECOVERY	13.00 (7-43)	13.00 (8-62)	Z_U=0.11 ^{NS}	.909

Table 2. DMSO: Dimethyl sulfoxide. RBC: red blood cells; PLT: platelets; ANC: absolute neutrophil count.

Graft recovery and need for blood transfusions

The results for these variables are shown in Table 2. The median number of days until leukocyte recovery was one day shorter in the 5% DMSO group when compared with the 10% DMSO group, although not statistically significant. Leucocyte engraftment was slightly shorter in the DMSO 5% group (14 days) than in the DMSO 10% group (15 days), thus reducing the duration of deep neutropenia; however, this difference failed to reach statistical significance. Two patients in the DMSO 10% group had delayed neutrophile engraftment attributed to infectious complications. For days until platelet recovery, the median was the same in both groups (13 days). These results are consistent with those previously reported[14]. The number of red blood cell concentrates and platelet pools transfused during admission in the post-implant period were similar. The median of transfused red blood cell concentrates was 4 in the 5% DMSO group when compared with 3 in the 10% DMSO group (p = 0.85). The median platelet transfusions were 2.5 and 3 in the 5% and 10% DMSO groups, respectively (p = 0.441).

Adverse effects

The analysis of adverse reactions observed during product infusion is shown in Table 3. In all patients, the overall rate of adverse reactions observed was 7.5% (n = 18). In the 5% DMSO group, the rate of adverse reactions was only 4.6% (n = 5) when compared with 9.8% (n = 13) recorded in the 10% DMSO group (p = 0.127). On analyzing the severity of reactions, a higher proportion of mild reactions (grade 1) was observed in the 10% DMSO group than the 5% DMSO group (5.6% *vs*. 4.6%, respectively). No cases of severe adverse events (grades 3-4) were observed in the 5% DMSO group, and 6 cases were recorded in the 10% DMSO group (p = 0.063). In particular, on comparing only cases with adverse reactions in both groups (n=18), significance was not established owing to the small sample size, but the size of the effect (19.3%; large) confirmed the relationship. The most frequent mild (grade 1) reactions were chills, pruritus, throat sore, bradycardia, and cough. Two patients presented a fever, two had shortness of breath needing oxygen, one patient suffered a hypertensive crisis, and another experienced seizure during infusion. This toxicity profile was similar to that described previously by other authors[5]. The higher frequency and severity of adverse reactions observed with higher DMSO concentrations were also observed in our population[13].

Table 3: Adverse effects

	Total (N=240)	**% (frequency)**		**Contrast test**	
		DMSO 5% (n=108)	DMSO 10% (n=132)	Value	P
ANY ADVERSE EFFECT				Chi^2= 2.33 ^{NS}	.127
-Yes	7.5% (18)	4.6% (5)	9.8%(13)		
-No	92.5% (222)	95.4% (103)	90.2% (119)		
SEVERITY OF REACTIONS					
Moderate/severe	2.5% (6)	0.0% (0)	4.5% (6)	Chi^2= 3.46 ^{NS}	.063
-Mild	5.0% (12)	4.6% (5)	5.3% (7)		

Table 3. DMSO: Dimethyl sulfoxide.

DISCUSSION

Although morbidity associated with DMSO is well known, it remains the standard cryoprotectant used in hematopoietic progenitor freezing. In recent decades, many centers have directed their efforts to establish strategies to reduce toxicity; however, several strategies have demonstrated cellular damage to the product with deleterious impacts on the graft. In the present study, the best results were obtained by decreasing the cryoprotectant concentration. Numerous studies have been designed to compare different concentrations of DMSO, to determine the minimum effective concentration that ensures the protection and survival of hematopoietic progenitors during freezing while minimizing adverse reactions in patients[14].

Despite most studies supporting the safety and efficacy of lowering the DMSO concentration, to date, many centers continue using 10% DMSO routinely. Although there is evidence supporting these results and some international cooperative groups encourage its standardization, no formal recommendations are available in guidelines[13].

Based on our experience with a large number of patients, and based on data published so far, we can conclude that reducing the DMSO concentration from 10% to 5% is safe for the patient and the product. This strategy reduces the undesirable and potentially serious effects of the cryoprotectant during infusion and improves cell viability of the product after thawing, without significantly impacting the time for engraftment or post-transplant hemoperipheral recovery[25].

Despite the limitations of our retrospective study (new protocol of apheresis, different conditioning regimens, different volume of infused products in both groups etc.), to the best of our knowledge, this is one of the few studies that evaluated this strategy in a higher number

of patients, revealing a statistically significant advantage in terms of post-thawing cell viability with a lower DMSO concentration.

Although the reduced rate of adverse reactions between the two groups did not reach statistical significance owing to the small number of cases observed in our population, this difference is clinically relevant.

Following the improvement of viability and absence of severe adverse infusion reactions in the last five years of clinical practice, we recommend adopting cryopreservation with 5% DMSO as the standard strategy. We believe that this cryopreservation option has clear benefits without increasing technical, economic, or staff resources, and can be easily adopted at all centers. Nevertheless, more prospective studies are needed to support these results and establish recommendations with a higher level of evidence.

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