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A faster reconstitution of hematopoiesis after autologous transplantation of hematopoietic cells cryopreserved in 7.5% dimethyl sulfoxide if compared to 10% dimethyl sulfoxide containing medium ☆

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ABSTRACT

Our previous *in vitro* studies proved a higher clonogenic potential of peripheral blood progenitor cells cryopreserved in 7.5% dimethyl sulfoxide (Me_2SO) than in 10% Me_2SO containing medium. Based on this findings 7.5% Me_2SO cryopreservation medium was introduced to our protocol and both the hematopoietic recovery and infusion-related toxicity were compared with that obtained with standard 10% Me_2SO containing solution. Two cohorts of consecutive patients treated with autologous hematopoietic stem cell transplantation were included in the analysis: 56 patients with PBPCs cryopreserved in 7.5% Me_2SO solution and 52 patients who obtained cells cryopreserved in 10% Me_2SO. Both study groups did not differ significantly with regard to age, diagnosis, and the number of transplanted $CD34^+$ cells. The time to leukocyte recovery was shorter for patients in the 7.5% Me_2SO treated group than in the 10% one. Reconstitution of platelets and the frequency of adverse events did not differ in both groups. Reduction of Me_2SO concentration from 10% to 7.5% in cryoprotective mixture has a beneficial impact on leukocyte recovery. These findings require verification in a prospective, randomized trial.

Introduction

Autologous peripheral blood progenitor cell (PBPC) transplantation is a well-recognized method for the treatment of various hematologic malignancies, particularly for multiple myeloma and lymphomas. PBSCs are obtained by leukapheresis, submitted to programmed cryopreservation and stored in liquid nitrogen (cryopreservation) until transplantation. Nevertheless, so far there have been no standards concerning composition of cryoprotective media and cryopreservation procedures. The basic constituent of these mixtures, necessary to secure cell viability, is dimethyl sulfoxide (Me_2SO). However, the concentrations of this cryoprotectant vary strongly between protocols in use ranging from 2.2% to 20% [18]. It is known that Me_2SO infused with transplant material may cause side effects including nausea, vomiting, dyspnea, skin flushing, cardiac dysfunction, and anaphylaxis. Severe neurologic toxicities were also reported by some investigators [10]. Intensity of side effects is believed to be dependent from volume of infused Me_2SO. Therefore, a reduction of the Me_2SO volume infused during transplantation is postulated to minimize adverse effects [4,8,12,16,18]. Washing away cryoprotective mixture before transfusion is possible, but it makes the procedure more complicated and may cause a loss of cells [6]. Another strategy is to decrease Me_2SO concentration, which raises concern regarding viability of PBSCs. Attempts to reduce Me_2SO concentration have been made by many investigators [1,9,17].

At the Department of Bone Marrow Transplantation and Oncohematology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Gliwice we performed an *in vitro* study, in which samples from the leukapheresis product were cryopreserved with various Me_2SO concentrations (2.5–10%) [14]. Although reduction of Me_2SO concentration was associated with decreased nucleated cell recovery, we demonstrated that the highest clonogeneic potential was obtained with samples cryopreserved with 7.5% Me_2SO. Following these findings we modified our clinical

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procedure by reducing the Me_2SO concentration in the cryoprotective medium from 10% to 7.5%. The goal of the current study was to evaluate retrospectively the consequences of the above modification with regard to the frequency of infusion-related adverse events and the time of hematopoietic recovery.

Materials and methods

Patients

Between Jan 2012 and Aug 2012, 56 consecutive patients were transplanted with autologous PBPCs cryopreserved in 7.5% Me_2SO solution (median age 57 years, range: 21–69). The historical control consisted of 52 patients, treated with transplantation of PBPCs cryopreserved in 10% Me_2SO (median age 55 years, range: 21–66) in a preceding period (Jun 2011–Jan 2012).

Both study groups did not differ significantly with regard to the diagnosis (mostly lymphoproliferative disorders), disease status at transplantation or conditioning regimen (chemo- or radiotherapy-based) (Table 1). All received granulocyte-colony stimulating factor (G-CSF, filgrastim) starting from day +7 after transplantation to support neutrophil recovery. Platelet transfusions were indicated when the platelet level dropped below 20×10^9/L while packed red blood cell transfusions were administered to maintain hemoglobin level >8 g/dl.

According to the institutional guidelines patients were reinfused all collected $CD34^+$ cells if a single transplantation procedure was planned or half of the material in case of planned double transplantation. The number of transplanted $CD34^+$ cells was comparable: median 7.5×10^6/kg (range 1.5–24.7) for 7.5% Me_2SO and 6.5×10^6/kg (2.1–24.6) for 10% Me_2SO group, $p = 0.68$. Determination of $CD34^+$ number was performed as described previously [14].

The study was approved by the local Bioethical Committee.

PBPCs collection and cryopreservation

The PBPCs were collected using Spectra-Optia Apheresis System (CaridianBCT Inc, Lakewood, CO, USA) according to the manufacturer's protocols, processing 2 blood volumes. Immediately after leukepheresis, cell suspensions were diluted by autologous plasma and 5% human albumin. Finally, pre-diluted Me_2SO solution was slowly added to the final concentration of 10% or 7.5% Me_2SO. During preparation the temperature was stabilized using ice-cooler. The final cell concentration in freezing bags was about 100×10^9/L. The suspension was aliquoted into 100 ml freezing bags, cryopreserved in controlled-rate IceCube 14 M freezer (Sy-Lab, Neupurkersdorf, Austria) and stored in liquid nitrogen vapor. Controlled-rate programs were separately optimized for 10% Me_2SO and 7.5% Me_2SO.

Table 1
Patient's characteristics.

	10% Me_2SO	7.5% Me_2SO	*p*
N	52	56	
Age (years)	55 (21–66)	57 (21–69)	0.88
Diagnosis			
MM	24 (46%)	25 (45%)	1.0
HL/NHL	23 (44%)	30 (54%)	0.34
Other	5 (10%)	1 (2%)	0.1
No. preceding lines of chemotherapy[a]	2 (1–4)	2 (1–4)	0.72
No. preceding cycles of chemotherapy	8 (3–23)	8 (1–30)	0.84
Preceding radiotherapy	19 (37%)	17 (30%)	0.54
Conditioning regimen			
Radiotherapy-based	34 (65%)	26 (46%)	
Chemotherapy-based	18 (35%)	30 (54%)	
No. transplanted $CD34^+$ cells ($\times 10^6$/kg)	6.5 (1.5–24.7)	7.5 (2.1–24.6)	0.68

Abbreviations: MM = multiple myeloma; HL = Hodgkin's lymphoma; NHL = non-Hogdkin's lymphoma.
[a] Line means repeated cycles of the same scheme of chemotherapy.

Monitoring of adverse effects and engraftment

The primary end-points of the study were: (1) the incidence of adverse events occurring within 24 h from the beginning of PBPC infusion, and (2) the time to leukocyte, neutrophil and platelet recovery to the threshold levels equaling 1.0×10^9/L, 0.5×10^9/L and 50×10^9/L, respectively. Toxicity of both procedures was assessed using Common Terminology Criteria for Adverse Events Version 4.0 [www://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf], definitions are listed in a subscript to Table 2.

Statistical methods

The frequencies of adverse events for the 7.5% and 10% groups were compared using Fisher's exact test, two sided. For comparison of the volume of infused Me_2SO U Mann–Whitney test was used.

Time of hematopoietic recovery was estimated using the Kaplan–Meier method. Two patients (one in each study group) did not reach platelet engraftment before discharge from the hospital and were included in the analysis as censored observations. The groups were compared with the use of log-rank test. In case of significant differences or tendencies, to verify the Kaplan–Meier results, a parametric method i.e., Weibull's regression has been applied [7] and the results were presented as hazard ratios with 95% credible intervals following both classical and Bayesian approach. The models were adjusted for age, the number of transplanted $CD34^+$ cells and conditioning regimen.

Table 2
Hematopoietic recovery after autoHSCT, need for transfusions and infusion-related complications according to Me_2SO concentration used for cryopreservation.

	10% Me_2SO	7.5% Me_2SO	*p*
N	52	56	
Me_2SO volume transfused (ml)[a]	30 (10–160)	22.5 (7.5–45)	0.02
WBC $>1.0 \times 10^9$/L recovery (median day, range)	11 (10–13)	11 (9–12)	0.03
ANC $>0.5 \times 10^9$/L recovery (median day, range)	11 (10–13)	11 (9–13)	0.04
PLT $>50 \times 10^9$/L recovery (median day, range)	12.5 (0–19)	12 (0–21)	0.36
RBC transfusions (median no., range)	0 (0–2)	0 (0–4)	0.27
PLT transfusions (median no., range)	1 (0–6)	1 (0–5)	0.2
PBPCs infusion-related complications (grade 1 or 2)[b]			
Nausea	15 (29%)	17 (30%)	1.0
Vomiting	4 (8%)	6 (11%)	0.74
Dizziness	1 (2%)	-	0.48
Weakness	-	1 (2%)	1.0
Any complication	20 (38%)	24 (43%)	0.7

Abbreviations: WBC = white blood cell count; ANC = absolute neutrophil count; PLT = platelets; RBC = red blood cells; PBPCs = peripheral blood progenitor cells.
[a] A single unit (100 mL) of transplant material containing 7.5 or 10 mL Me_2SO was infused in 10 min. The transplantations were done in a single session except for one patient in whom due to high amount of Me_2SO (160 mL) it was divided in two sessions in two consecutive days.
[b] According to Common Terminology Criteria for Adverse Events Version 4.0: Nausea grade 1 means loss of appetite without alteration in eating habits; nausea grade 2, oral intake decreased without significant weight loss, dehydration or malnutrition; vomiting grade 1, 1–2 episodes (separated by 5 min) in 24 h; vomiting grade 1, 3–5 episodes (separated by 5 min) in 24 h; dizziness grade 1, mild unsteadiness or sensation of movement; dizziness grade 2, moderate unsteadiness or sensation of movement; fatigue grade 1, fatigue relieved by rest; fatigue grade 2, fatigue not relieved by rest, limiting instrumental activity of daily leaving.