Indian J Hematol Blood Transfus (July-Sept 2019) 35(3):563–564
https://doi.org/10.1007/s12288-019-01140-7

CORRESPONDENCE

Mechanical Cryopreservation of Peripheral Blood Stem Cell: Initial Experience from a Tertiary Care Hospital

Nitin Agarwal[1] · Prashant Pandey[1] · Esha Kaul[2] · Nivedita Dhingra[2] · Praveen Kumar[1]

Received: 14 February 2019 / Accepted: 17 May 2019 / Published online: 28 May 2019

Sir,

Cell therapies based on hematopoietic stem cells (HSCs) have become the standard of care for a large number of clinical indications. These indications include lymphoma (Hodgkin's and non-Hodgkin's) other hematological malignancies, myelodysplastic syndromes, certain solid tumors etc. [1]. Stem cells can be stored at 2–6 °C for 72 h but for long term storage of stem cells, they need to be cryopreserved. Over the years, peripheral blood haematopoietic stem cells (PBSC) have been cryopreserved in liquid nitrogen at − 196 °C, in vapour phase nitrogen at − 152 °C or − 135 °C, and in mechanical freezers at − 80 °C [2]. Here, we report an analysis of first 10 cryopreservation of peripheral-blood stem-cell (PBSC) in mechanical freezers at our center in terms of CD34+ viability and cell engraftments in those patients.

PBSCs were collected from auotologus donors using P1YA kits on Com.tec (Fresenius kabi). A solution made up of 10% DMSO, 20% human serum albumin with 6% HES (Voluven, Fresenius Kabi, Germany) was used for cryopreservation of cells. An equal amount of cryoprotective solution was mixed to the PBSC and transferred into Cryocyte bags (Macopharma) that were placed into a − 80 °C mechanical freezer (Thermo Scientific, USA). Whole procedure was done under fully aseptic technique using laminar air flow. Stem Cells were thawed in 37 °C water bath and then infused within 15 min without washing. No post infusion growth factors were given to the patients. The criteria of engraftment were to reach count of 20×10^9/L for platelets and 0.5×10^9/L for neutrophils respectively.

All 10 patients were posted for re-transplant (2nd/3rd) and were heavily pre-treated with chemotherapy. 4 patients were of relapsed peripheral T cell lymphoma and 2 each of relapsed large B cell lymphoma, relapsed lymphoblastic lymphoma and stage 4 neuroblastoma. Average time duration of storage for cryopreserved stem cells was 27 days (range 10–56 days). The initial yield of the products before cryopreservation ranged from 3×10^6 to 10.2×10^6 per Kg of the patient (mean dose 7.85×10^6 per Kg) which decreased to a mean of 5.95×10^6 per Kg post thawing of frozen stem cells. Stem cells viability after thawing ranged from 77 to 90% of total CD34+ cells. All the patients had neutrophil and platelet engraftment before they were discharged. Days taken for engraftment of neutrophil ranged from 10 to 15 days (mean 11.5 days) whereas for platelets, it ranged from 14 to 43 days (mean 23.5 days) (Table 1).

We used the standard cryoprotectant DMSO, which prevents freezing damage to living cells. Varying percentages of DMSO has been used with success but 10% DMSO has been found to be the optimal one as higher percentage leads to more toxicity and lower concentration leads to suboptimal storage [3]. It was seen by Katayama, that to enhance the effect of the cryopreservative, the combination of DMSO and the extracellular cryoprotectant hydroxyethyl starch (HES) was more successful in PBSCs and bone marrow grafts [4]. In our study, we used a

✉ Nitin Agarwal
nuts_medico@rediffmail.com;
nitin1.agarwal@jalindia.co.in

Prashant Pandey
pkpandey2007@gmail.com

[1] Department of Transfusion Medicine, Histocompatibility and Molecular Biology, Jaypee Hospital, Sector-128, Noida 201304, India

[2] Department of Hemato-Oncology and BMT, Jaypee Hospital, Sector-128, Noida 201304, India

Table 1 Characteristics of PBSCs stored in mechanical freezers

Mean initial yield (per Kg)	7.85×10^6 (3×10^6–10.2×10^6)
Mean post thaw yield (per Kg)	5.95×10^6 (2.4×10^6–8.9×10^6)
Mean storage time	27 days (10–56 days)
Stem cell viability (range)	77–90%
Days for engraftment (neutrophils)	11.5 (10–15)
Days for engraftment (platelets)	23.5 (14–43)

solution made up of 10% DMSO, 20% human serum albumin with 6% HES for cryopreservation of cells.

It was Stiff et al. [5] in 1987 who first demonstrated the successful engraftment with bone marrow stem cells stored at − 80 °C after uncontrolled-rate freezing. Various studies have shown that there is no adverse effect on storage of PBSCs at − 80 °C and have reported no significant change in post-thawing recovery parameters including cell viability, CD34+ cells, burst forming unit, erythroid or colony forming unit-granulocyte macrophage.

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