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Effects of long-term cryopreservation on peripheral blood progenitor cells

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Abstract

Background aims. The long-term stability of cryopreserved peripheral blood progenitor cells is an important issue for patients experiencing disease relapse. However, there is no consensus on how to evaluate the long-term effects of cryopreservation. We describe the effect of cryopreservation on viability and progenitor colony activity from 87 individual samples processed at the Scripps Green Hospital Stem Cell Processing Center (La Jolla, CA, USA). *Methods.* We randomly selected 87 peripheral blood hematopoietic stem cell (PBHSC) samples from 60 patients and evaluated the effect of cryopreservation on sample viability and red and white cell colony activity after <24 h and 7, 10 and 15 years of cryopreservation. Viability was assayed via trypan blue dye exclusion and activity was measured following 14 days of culture. *Results.* An age at collection older than 50 years may result in suboptimal activity and viability following long-term cryopreservation, while gender and disease status had no effect. Cryopreservation did not significantly affect white or red cell activity following 10 years of cryopreservation. However, for samples stored longer than 10 years, viability and activity significantly decreased. We noted a positive association between higher pre-cryopreservation %CD34 count and colony activity. *Conclusions.* Cryopreservation of peripheral blood progenitor cells for up to 10 years results in no loss of clonogenic capacity, as determined by culture activity, although longer durations of storage may affect activity. Until validated methods are developed, cryopreserved grafts should be evaluated based on pre-freeze $CD34^+$ cell counts as assayed by flow cytometry, and post-thaw sample evaluation should be reserved for patients identified as poor mobilizers.

Key Words: *clonogenic assays, cryopreservation, hematopoietic stem cells, long-term storage, peripheral blood progenitor cells, viability*

Introduction

High-dose chemotherapy followed by autologous stem cell transplantation represents a standard of care for upfront and relapsed hematologic and non-hematologic malignancy (1–4). Long-term cryopreservation represents a means of holding a potential therapeutic modality in reserve for use at a future date (5), and previous reports have described preserved peripheral blood hematopoietic stem cell (PBHSC) viability for many years (5–10). Knowing the effect of cryopreservation on the functionality of such cells is of vital importance because the long-term preservation of progenitor cell components has become commonplace at many institutions (6).

Previous reports have described successful transplantations using cryopreserved hematopoietic stem cells (HSC) in the face of declining clonogenic capacity over time (8–10). Furthermore, many studies have described a mixture of PBHSC and bone marrow-derived hematopoietic stem cells (BMHSC), although often with conflicting conclusions regarding the effects of long-term storage on engraftment potential, as measured by the absolute number of $CD34^+$ cells recovered post-thaw and post-thaw progenitor cell colony assays (5,7,11). The clinical effect of these changes appear minimal because they relate to time to neutrophil and platelet engraftment (12,13). However, the incidence of adverse events

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and their significance is unclear, although most reports indicate that infusion of cryopreserved HSC is well tolerated (13–15).

We report on the effect of cryopreservation on PBHSC activity and viability from 85 PBHSC samples processed and stored for up to 15 years at the Scripps Green Hospital Stem Cell Processing Center (SGHSCPC; La Jolla, CA, USA), collected between 1995 and 2006.

Methods

Sample selection

We reviewed the SGHSCPC records and selected at random 87 unique PBHSC samples from 60 individual patients for evaluation. The samples were evaluated for viability and activity after less than 24 h cryopreservation (to assess the effect of the controlled-rate freezing procedure on PBHSC) and after 7, 10 and 15 years of cryopreservation. This project was reviewed and approved by the Scripps Health Office for the Protection of Research Subjects/Institutional Review Board (La Jolla, CA, USA).

Isolation of PBHSC

Following donor stimulation [(19.7% of samples mobilized with granulocyte colony-stimulating factor (G-CSF), 13.2% with cyclophosphamide and G-CSF, 61.8% with cyclophosphamide, G-CSF and granulocyte–monocyte colony-stimulating factor (GM-CSF), and 2.6% with cyclophosphamide, etoposide and G-CSF, while 2.6% of samples did not have data collected for the mobilization regimen], hematopoietic progenitor cell (HPC) apheresis [HPC (A)] products were collected via buffy coat isolation using a COBE® Spectra apheresis system (CaridianBCT, Lakewood, CO, USA). Each product was collected for 4 h using a high-flow/high-rate peripheral blood apheresis method. Heparin and anticoagulant citrate dextrose solution A (ACD-A) were both used as anticoagulants. $CD34^+$ cells were quantified by flow cytometry (Genoptix Inc., Carlsbad, CA, USA).

Freezing and storage process

Briefly, following collection, the HPC (A) products were tested and processed in preparation for cryopreservation. They were volume adjusted to yield a final cryopreserved cell concentration of $1.0–3.0 \times 10^8$ total nucleated cells (TNC)/mL, then chilled to 4°C. Chilled freezing media solution [10% dimethyl sulfoxide (DMSO; Edwards Lifesciences, Irvine, CA, USA)], 9.5% Plasma-Lyte with 5% dextrose (©Baxter, Deerfield, IL, USA) and 5.5% human serum albumin (Aventis Behring, King of Prussia, PA, USA) were added to the chilled product for cryopreservation. Then 50–70-mL aliquots, as well as 1–2 mL duplicate 'test' aliquots of the mixed cell/freezing media mixture, were transferred to 250-mL cryocyte freezing containers (©Baxter).

Cryocyte containers were placed into a CryoMed (©Thermo Fisher Scientific Inc., Waltham, MA, USA) liquid nitrogen (LN_2) controlled-rate freezer and cryopreserved at a rate of 1°C/min until a temperature of −90°C was attained. Cryopreserved PBHSC were then transferred to a Forma Scientific CryoPlus® (©Thermo Fisher Scientific Inc., Waltham, MA, USA) storage freezer in the liquid/vapor phase of LN_2 at ≤ -130°C. All test aliquots were stored in the vapor phase. Clonogenic assays were performed on test aliquots only.

Determining viability

One-hundred microliters of fresh (at the initial date of collection, representing baseline values for each variable at each time-point) or thawed PBHSC product were combined with 900 μL Medium 199 (©Baxter); 10 μL of this mixture were added to 10 μL of a 0.4% trypan blue solution and transferred to a hemacytometer. Nucleated cells were counted in a $1 \times 1 \times 0.1$-mm area (corresponding to four large squares on the hemacytometer). 'Live' nucleated cells were clear and refractile while 'dead' nucleated cells were stained blue. The percentage nucleated cell viability was calculated as follows: number of live cells/(number of live cells + number of dead cells) $\times 100 =$ %viability (16).

Determining activity/PBHSC culture

PBHSC cultures were prepared as described in the *Human Colony-Forming Cell Assays Using MethoCult*® technical manual from StemCell Technologies (©Stem Cell Technologies Inc., Vancouver, Canada) (16). Briefly, 400-μL aliquots of PBHSC were removed from the post-processing product for culture. The nucleated cell concentration was adjusted to 25×10^6 cells/mL using Medium 199, and 5 μL of this mixture were transferred into 2.5 mL of MethoCult GF H4434 (©Stem Cell Technologies, Inc.) media to yield a final cultured concentration of 5×10^4 nucleated cells/mL. The inoculated MethoCult GF H4434 (©Stem Cell Technologies Inc.) was then transferred as duplicate 1-mL aliquots to Petri dishes and incubated at 37°C in 5% CO_2 for 14 days (16).

PBHSC activity was determined by counting the total number of white blood cell and red blood cell colonies on culture plates. White cell colonies (WCC) represented the progenitor populations of