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Evaluation of trehalose and sucrose as cryoprotectants for hematopoietic stem cells of umbilical cord blood ☆

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Abstract

Bone marrow transplantation (BMT) is a therapeutic procedure that involves transplantation of hematopoietic stem cells (HSC). To date, there are three sources of HSC for clinical use: bone marrow; mobilized peripheral blood; and umbilical cord blood (UCB). Depending on the stem cell source or type of transplantation, these cells are cryopreserved. The most widely used cryoprotectant is dimethylsulfoxide (Me_2SO) 10% (v/v), but infusion of Me_2SO-cryopreserved cells is frequently associated with serious side effects in patients. In this study, we assessed the use of trehalose and sucrose for cryopreservation of UCB cells in combination with reduced amounts of Me_2SO. The post-thawed cells were counted and tested for viability with Trypan blue, the proportion of HSC was determined by flow cytometry, and the proportion of hematopoeitic progenitor cells was measured by a colony-forming unit (CFU) assay. A solution of 30 mmol/L trehalose with 2.5% Me_2SO (v/v) or 60 mmol/L sucrose with 5% Me_2SO (v/v) produced results similar to those for 10% (v/v) Me_2SO in terms of the clonogenic potential of progenitor cells, cell viability, and numbers of $CD45^+/34^+$ cells in post-thawed cord blood cryopreserved for a minimum of 2 weeks. Thus, cord blood, as other HSC, can be cryopreserved with 1/4 the standard Me_2SO concentration with the addition of disaccharides. The use of Me_2SO at low concentrations in the cryopreservation solution may improve the safety of hematopoietic cell transplantation by reducing the side effects on the patient.

Keywords: Cryopreservation; Dimethylsulfoxide; Hematopoietic stem cells; Sucrose; Trehalose

Hematopoietic stem cell transplantation (HSCT) is a medical therapeutic procedure which aims to reconstitute the hematopoietic activity of bone marrow. It is the first treatment option for many bone marrow diseases such as bone marrow aplasia or leukemia, and an adjuvant tool in the treatment of solid tumors. As an adjunct in the treatment of malignancies, HSCT offers hope in cancer treatment as new and more aggressive therapies are used [6]. Stem cells from bone marrow (BM), mobilized peripheral blood (MPB) or umbilical cord blood and from different donor types (autologous, syngeneic or allogeneic) are used for these transplants [32]. Often these transplant modalities require cryopreservation and storage of hematopoietic stem cells (HSC). This is especially true of umbilical cord blood stem cells, which need to remain cryopreserved in umbilical cord blood banks for possible future use.

Umbilical cord blood (UCB) is a valuable source of stem cells in the treatment of hematologic, oncologic, immunologic and metabolic diseases [4,12,20]. Most UCB units are stored in liquid nitrogen in individual bags for several years and quickly thawed just prior to transplantation. A single unit contains a limited number of hematopoietic progenitors, although with high proliferation and repopulating

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capabilities [33,34]. UCB transplant provides the advantages of requiring less stringent HLA matchings between donors and recipients as well as a lower severity of graft versus host disease (GVHD) after transplant. However, it is associated with higher morbidity and mortality due to delayed neutrophil (median >25 days) and platelet (median >80 days) engraftments, essentially as a result of the limited dose of HSCs in UCB when compared to transplants using BM or MPB as HSC sources [20,19,29].

Optimization of cryopreservation protocols to maintain the quality of HSCs has been an important task for UCB banks. To allow this prolonged storage, the HSC product is slowly cooled at a controlled rate and stored at −196 °C in liquid nitrogen. Cooling the cells slowly avoids intracellular ice buildup, which can cause rupture of the cell membrane. Nevertheless, it can result in dehydration of the cells by formation of extracellular ice. To prevent this, a cryoprotectant is added. The most widely used cryoprotectant is dimethylsulfoxide (Me_2SO) that is a hygroscopic polar compound developed originally as a solvent for chemicals. Its properties were described originally in 1959 by Lovelock et al. [16].

Cryopreserved HSC products can be stored for years [30]. The presence of cryoprotectant and changes resulting from the freezing and thawing process require special precautions during and after the infusion of HSC product into the patient [25].

Several side effects have been described during the infusion of Me_2SO into patients, including sedation, headache, nausea, vomiting, hypertension, bradycardia, hypotension or anaphylactic shock [26]; effects on the blood include intravascular hemolysis, hyperosmolality, and increased serum transaminase levels after IV administration in humans [22,23,35].

Disaccharides such as sucrose and trehalose have been widely used as natural cryoprotectants [17]. In nature, many organisms can survive in an inert, desiccated state for extended periods with up to 99% water loss, in a phenomena known as anhydrobiosis [9,18], which is much similar to the phenomena that occurs during cryopreservation and related in some instances to the accumulation and storage of saccharides, such as trehalose. Trehalose may also be used in parenteral nutrition. Moreover, the combination of catalase and trehalose as a supplement in conventional freezing medium resulted in better protection of growth factor receptors, adhesion molecules, and functionality of hematopoietic cells [24,36].

In this study, we assessed the ability of two disaccharides, trehalose and sucrose, to enhance the cryopreservation of UCB. Both sugars enabled the concentration of Me_2SO to be lowered in the infusion product of the HSCT.

Materials and methods

Umbilical cord blood

Umbilical cord blood units were obtained according to the local procedures of the UCBB of the National Cancer Institute (INCA-Brazil). All procedures were approved by the institutional ethics committee.

UCB collection and processing

Pregnant women were recruited by interview to donate their child's cord blood before delivery. Potential donors were screened with a clinical questionnaire in the maternity hospital. UCB units were collected from eligible mothers with an *ex-utero* procedure by the nurses from the UCBB working in the maternity hospital for that purpose. Briefly, after natural delivery of the placenta, the umbilical cord and placenta were sent to the collection room where the placenta was hung up, allowing the umbilical cord to be cleaned. After this procedure, the cord blood was collected by gravity into blood collection bags, containing CPDA-1 anticoagulant solution, by insertion of a needle into the umbilical vein. After collection, the bag was sealed and sent to the processing facility of the INCA Cord Bank. Before processing, cells were counted in a sample of UCB using a Cell-Dyn 1700 (Abbott Ltda, Sao Paulo, SP, Brazil) and then the sample was mixed with hydroethylstarch in a 4:1 ratio and centrifuged at 59*g* for 8 min. The white blood cell (WBC)-rich plasma was collected in a separate bag and centrifuged at 500*g* for 12 min [14]. UCB was processed up to 24 h from delivery of the placenta.

Cell lines

The cell lines KG1, K562 and S17 were obtained from the Rio de Janeiro Cell Bank (APABCAM, Rio de Janeiro, RJ, Brazil) and maintained in our lab according to standard protocols, in RPMI medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (Invitrogen, Carlsbad, CA, USA) and L-glutamine (Sigma–Aldrich Corp., St. Louis, MO, USA) 1 mmol/L.

Cryopreservation and thawing

In a pilot study, the best concentrations of Me_2SO, sucrose, trehalose, and their combinations were determined using hematopoietic cells lines KG1, K562 and bone marrow stromal cell line S17. Forty-three different combinations of cryoprotectants were tested with the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) viability assay, and 10 combinations were chosen for use with UCB cells. A Me_2SO 10% (v/v) solution was used as the standard cryopreservation solution (100% viability) and an aliquot of cord blood cells without any cryoprotectant was used as a negative control (0% viability).

Preparation of cryoprotectant solutions

All solutions were prepared in previously marked cryovials just prior to use and before addition of cells. After addition of the cells, all the vials had 1 mL of final suspension. Dextran 40 (Fresenius KABI, Rio de Janeiro, RJ,