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Hydroxyethyl starch as a substitute for dextran 40 for thawing peripheral blood progenitor cell products

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Abstract

Background aims. Removing DMSO post-thaw results in: reduced infusion reactions, improved recovery and stability of viable CD34+ cells. Validated methods use 5%–8.3% Dextran 40 with 2.5%–4.2% HSA for this purpose. Recent shortages of clinical grade Dextran require identification of suitable alternatives. *Methods.* PBPC were used to compare a standard 2X wash medium of 5 parts 10% Dextran 40 in saline (DEX) with 1 part 25% HSA (8.3% DEX/ 4.2% HSA) with Hydroxyethyl Starch (HES)-based solutions. Cells in replicate bags were diluted with an equal volume of wash solution, equilibrated 5 minutes, the bag filled with wash medium, pelleted and the supernatant expressed. Bags were restored to the frozen volume in wash medium and tested by single platform flow cytometry and CFU. Total viability, viable TNC, MNC, and CD34+ cell recovery, and CD34+ cell viability were compared immediately post-thaw and after 90 minutes. *Results.* 5.2% HES/4.2% HSA did not differ from our standard in CD34 recovery or viability. Due to concerns that high concentrations of HES could affect renal function we tested 0.6% HES/2.5% HSA resulting in significantly poorer CD34 recovery and viability. Results improved using 2.4% HES/4.2% HSA and when 0.6% HES/4.2%HSA was used no significant differences were seen. CFU assays confirmed no differences between the standard dextran arm and HES at 2.4% or 0.6% so long as HSA was at 4.2%. *Conclusions.* We conclude that HES from 0.6% to 5.2% with 4.2% HSA is a suitable substitute for Dextran 40 as a reconstitution/washing medium for PBPC products.

Key Words: *cryopreservation, thawing, low molecular weight dextran, dextran 40, hydroxyethyl starch*

Introduction

Autologous cellular therapy products are commonly cryopreserved to allow time for preparative regimens to be administered. Hematopoiesis is restored by infusion of the stored cells after clearance of preparative regimen drugs from the circulation. Although not as common, products from allogeneic donors may also be cryopreserved for logistical reasons or when cells in excess of those needed to restore hematopoiesis have been collected. The exact methods used for cryopreservation and thawing vary, and there is no clearly defined optimal method for all hematopoietic progenitor cell (HPC) product types [1,2]. Studies performed by Rubenstein et al [3] during the early days of cord blood banking showed improved recovery of viable $CD34^+$ cells when thawed cells were first diluted and then washed with a solution of 10% dextran 40 in saline mixed equally with 5% human serum albumin (HSA). The wash medium was used to return the cells to their original cryopreserved volume for infusion. This procedure removed red blood cell (RBC) stromal, dead cells and other cellular debris and reduced the clumping typically seen in unwashed products. The wash procedure was later modified to mix five parts of 10% dextran 40 with 1 part 25% HSA to yield a solution with 8.3% dextran 40/4.2% HSA that has become standard at most cord blood banks [4]. Subsequent studies have shown that simple dilution of RBC reduced cord blood products in dextran-based solutions was equally effective in preserving $CD34^+$ cells post-thaw [5]. However, because of reports of serious adverse events, including death, associated with the use of unwashed, RBC-replete cord blood units, a dextran/albumin wash method is now required by standards [6] and is

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recommended by experts in the field [7] for RBC-replete units.

Although initially used for cord blood, dextran/albumin wash methods were also useful in thawing other HPC products, including bone marrow buffy coat fractions that contain significant RBC stroma [3] and peripheral blood progenitor (PBPC) products [1,8,9]. The incidence of infusion reactions to non-washed thawed products in adults has been reported to vary widely from 8% to 82%, and although mostly mild, serious, even life-threatening events have been reported [8]. At our own center, a review of 1188 (89% PBPC, 4% marrow and 7% cord blood products) washed product infusions over the past 10 years showed an infusion reaction rate of only 5.1%, most of which were hypertension and none of which were serious. The post-thaw stability of viable $CD34^+$ cells in thawed washed products is significantly prolonged, allowing for thawing in the laboratory resulting in a better ability to troubleshoot potential problems such as fractured or leaking bags [10].

The basis for improved recovery of $CD34^+$ cells using dextran/albumin wash methods is believed to be a decrease in osmotic shock as dimethyl sulfoxide (DMSO) leaves the cell membrane in the presence of these solutions. Over the past 2 years, there have been periodic shortages of 10% dextran 40 in saline on the market, and, to a lesser extent, dextran 40 in 10% dextran has also been affected. These shortages reached acute proportions during this past year, requiring that alternatives to dextran 40 in saline be identified if the advantage of using washed products was to be maintained. Here we report a series of experiments, first to verify solutions of 10% dextran 40 in dextrose and then to validate use of hydroxyethyl starch (HES) as alternatives to 10% dextran 40 in saline for washing thawed PBPC products.

Methods

Products

Products used in this study were cryopreserved at final cell concentrations of $\leq 4.0 \times 10^8$ cells/mL in DMSO (10%), 25% HSA (16%, equals 4% HSA) and 24% isotonic electrolyte solution, either Plasmalyte A or Normosol R. Products ($n = 34$) were approved for discard according to agreements in place at collection. All products were controlled-rate frozen in two or more bags of equal content such that each experiment had its own paired control.

Wash solutions

The standard wash solution, except where indicated, was prepared from 10% dextran 40 in saline (Hospira) (five parts) diluted with 25% HSA (one part) to contain 8.3% dextran with 4.2% HSA. Test solutions used 10% dextran 40 in 5% dextrose (Hospira) or 6% HES in saline (Hespan, Braun Medical, Inc.). HES was diluted with isotonic electrolyte solution to achieve the indicated concentration. Wash solutions were chilled to 1–6°C after preparation.

Wash procedure

The primary bag was removed from its cassette and overwrap and placed in a sterile zip-lock bag. The sealed, air-free bag was placed in a 37°C water bath and thawed with gentle kneading until only a few ice crystals remained. The product was transferred to a biosafety cabinet and removed from the zip-lock bag. Thawing solution equal to the product volume was slowly added with gentle mixing and cells allowed to equilibrate for 5 min. Cells were then transferred to a 300-mL transfer pack that was filled with additional chilled wash solution and centrifuged at 514 *g* for 15 min at 10°C. Bags were transferred to the biosafety cabinet, placed in a plasma extractor, connected to a new transfer pack, and supernatant was expressed to the level just above the cell pellet. Cells were restored to their original cryopreservation volume, mixed, and sampled for end of washing testing. The bag was placed at 1–10°C before a second sampling 90 min post-washing. For each paired experiment, products were thawed at the same time by different technologists using separate equipment and wash solutions.

Testing

In all experiments product samples were tested for total nucleated cell (TNC) count using a hematology analyzer (AcT2 diff, Coulter). Flow cytometry was used to assess overall viability, $CD34^+$ cell viability and viable recovery of $CD34^+$ cells, TNCs and mononuclear cell (MNC) count. All flow studies were performed using a single platform, no wash and no lyse method as originally described by Sartor *et al.* [11]. Testing was repeated after 90 min. Cell recoveries were based on values at cryopreservation and CD34 viability was determined by analyzing both 7-aminoactinomycin D (7-AAD) negative (viable) and positive (dead) populations. Some experiments included assessment of colony forming units (CFU) using MethoCult Classic medium (Stem Cell Technologies) by a modified plate method CFU assay [12]. CFU cultures were plated in duplicate at Medical College of Wisconsin and after inoculation into MethoCult tubes and transport to Duke University Medical Center.