PAS-C PLATELETS CONTAIN LOWER SUPERNATANT ISOHEMAGGLUTININ TITERS AND HLA ANTIBODY SPECIFICITIES AND INCREASED SOLUBLE CD40 LIGAND VERSUS PLASMA PLATELETS

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INTRODUCTION/ABSTRACT

Platelet additive solution 3 (PAS-3, Intersol, Fresenius Kabi USA, Lake Zurich, IL) – a balanced salt solution – may be used to replace approximately two thirds of human plasma for the storage of apheresis platelets collected on the Amicus separator (Fresenius Kabi USA, Lake Zurich, IL). Platelets stored in 65% PAS/35% plasma (PAS-C platelets) maintain functional integrity for up to 5 days, and reduction in donor plasma may be beneficial for platelet recipients 1. PAS-C platelets are presumed to reduce recipient exposure to donor plasma components; however, the effects of PAS-C on platelet supernatant composition are poorly defined.

OBJECTIVES

To better define the effects of PAS on platelet supernatant composition, we compared the following factors in supernatants of PAS-C platelets to plasma platelets.

• Total protein
• Isohemagglutinin titers
• HLA antibodies
• In-vitro neutrophil priming activity

MATERIALS AND METHODS

Sample collection. The study comprised 100 SDPs collected by apheresis from consecutive group O donors at the New York Blood Center with 50 collected into 100% plasma (PAS-3A) and 50 collected into 65% PAS/35% plasma (PAS-C). After collection, a 5 mL sample was withdrawn from each unit, the platelets were pelleted and the supernatant was withdrawn and frozen for further testing.

Total protein measurement. Total protein was measured using a modified bicinchoninic acid assay on a Dimension Vista analyzer (Siemens, Munich, Germany).

Isohemagglutinin titers. Anti-A and anti-B isoagglutinin titers were determined by serologic tube testing with straight and serial two-fold dilutions of platelet supernatant. In each reaction, 100 µL of straight or diluted SPD supernatant was added to 50 µL of type A1 or B2 red blood cells (Ortho Clinical Diagnostics, Raritan, NJ), and the samples were read for direct agglutination upon immediate spin. For each SPD supernatant sample, testing was performed with straight supernatant and increasing serial two-fold dilutions of SPD supernatant into 0.9% saline until agglutination was no longer detected. The titer was defined as the highest dilution that produced at least 1+ agglutination.

HLA antibody assessment. Screening for HLA antibodies was performed using the One Lambda (Canoga Park, CA) LabScreen mixed Luminex assay. Samples which exceeded the positive threshold were further tested with single antigen beads (LABScreen, One Lambda Inc.) to identify Class I and II antibody specificities.

Neutrophil isolation and priming. Neutrophils (PMNs) were isolated from heparinized whole blood after informed consent was obtained from healthy volunteer donors under an approved protocol by Colorado Multi-Institution Review Board at the University of Colorado Denver by standard technique using dextran sedimentation, ficoll-paque separation, and hypotonic lysis, as previously described 2. PMNs (3.5 x 10⁵ cells) were incubated in Krebs Ringer Phosphate with Dextrose, pH 7.35 (KRPL) buffer along with supernatant or lipid samples (10%) for 5 min at 37°C then activated with 1µM formyl-Met-Leu-Phe (fMLF) and the superoxide dismutase inhibitable maximal rate of superoxide (O₂⁻) was measured at 550 nm, as previously reported 2. Priming activity was measured as the augmentation of the maximal rate of superoxide production by PMNs activated with fMLF.

Lipid Extractions. Lipids were isolated from the plasma samples using a 1:1:1 chloroform:methanol:water:0.2% acetic acid extraction, as previously published. 18 Lipids were then solubilized with 1.25% fatty acid free human serum albumin and used to assess in PMN priming activity, as previously reported 2, 3.

Statistics. The data is presented as the mean with 95% confidence interval (CI) or the median with the range from 25th percentile to 75th percentile depending on the data analyzed. Statistical analysis and data visualization was performed using Prism software with paired t-tests employing the Mann-Whitney U test or Fisher’s exact test depending upon the data analyzed (GraphPad, San Diego, CA).

RESULTS

Observational studies show decreased allergic and febrile non-hemolytic transfusion reactions in recipients of PAS-C platelets compared to plasma platelets 1. These effects are attributed to dilution of plasma protein antigens, and cytokines. The supernatant of PAS-C platelets is expected to contain 35% of the total protein concentration of conventional plasma platelets. We measured the total protein in supernatant of plasma and PAS-C SDPs.

Plasma from group-O platelet donors may contain high levels of anti-A and anti-B isoagglutinins, and replacing the donor plasma with 65% PAS-C is expected to decrease anti-A and anti-B isoagglutinin titers, which may prevent hemolysis after transfusion of ABO incompatible platelet units. To quantify the degree to which isoagglutinin titers are reduced in PAS-C compared to plasma platelets, titration for direct agglutination at immediate spin was performed on A and B red cells using supernatant from conventional plasma and PAS C SDPs from group O donors.

Total supernatant isoagglutinin titers were determined as the function of treatment group: PAS C and plasma SDPs (n=50 for each group). The line indicates the mean and bars indicate the 95% confidence interval.

CONCLUSIONS

Decreased plasma proteins likely underlie lower rates of allergic and febrile non-hemolytic transfusion reactions seen with use of PAS-C platelets. Decreased anti-A and anti-B titers may prevent hemolysis from minor ABO mismatch. Lower HLA-antibody specificities may mitigate transfusion related acute lung injury (TRALI), increased PMN priming by PAS-C platelets is likely due to platelet membrane release of cSD40L and not bioactive lipids. Although cSD40L has been associated with TRALI, only PMN priming with lipid - not cytokine - agents has been causally linked with TRALI. The mechanism and clinical impact of increased cSD40L in PAS-C platelets remain to be elucidated.

REFERENCES


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