BECKMAN COULTER

Platelet Function By Flow Cytometric Analysis

Lori A. Charie, Janet Chinners, and Rhonda Mills
Biomedical Research, Beckman Coulter, Inc.

Abstract

The role of flow cytometry in platelet function studies continues to increase as the method offers significant advantages in sensitivity. Of particular interest is the role of platelet activity and reactivity in disorders such as acute coronary syndromes and thrombotic events. Additionally, the presence and quantity of platelet-leukocyte aggregates has been associated with disease activity. In order to assess innate platelet reactivity and aggregates as well as the platelet activation potential, a multi-color flow cytometric assay was performed using markers for activation (CD62p), aggregation (CD31), tissue factor (CD142), and gating antigens (CD45 and CD41). Color compensation was performed using the full matrix method. To assess platelet activation potential, specimens were drawn into sodium citrate or directly into ThromboFix™, a platelet stabilizer. The citrate specimens were further activated with PMA, TRAP, ADP, and collagen that had been titered to produce some activation yet maintain a platelet count sufficient to analyze. Platelet activation was evaluated by CD62p expression. The platelet activation potential was defined as the difference in CD62p expression between unactivated, stabilized platelets and activated platelets. Variable responses were observed among donor specimens with respect to CD62p percent positivity and mean fluorescence intensity for all activation agents although PMA produced the most uniform response. CD31, an aggregation marker, did not exhibit any significant differences regardless of activation or activator. Levels of CD142 (tissue factor) appeared to be elevated on the PMA-activated CD45 positive populations. The number of platelet-leukocyte aggregates also appeared to vary in specimens that had been activated with PMA or ADP.

Introduction

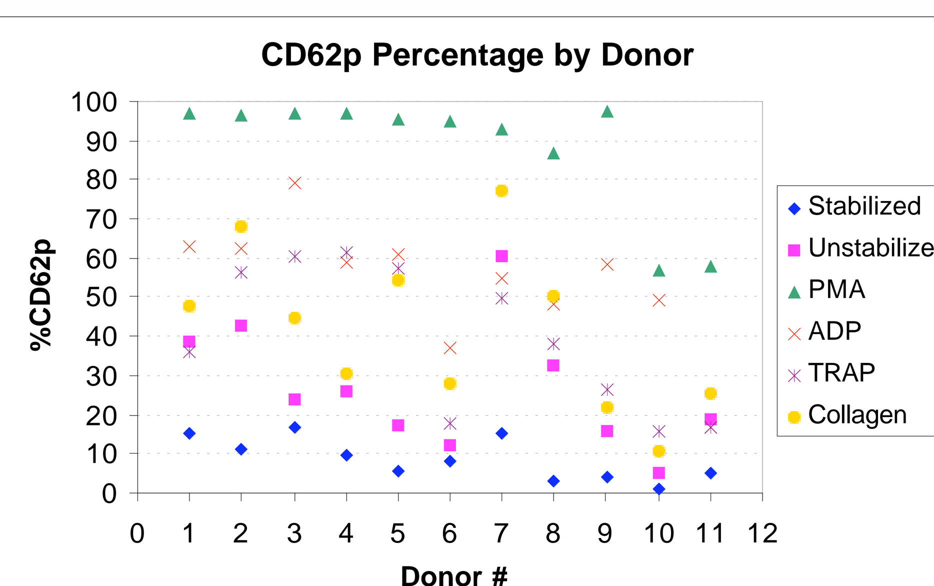
- While platelet activation is a critical mechanism in the hemostasis process, hyperreactivity may be implicated in thrombotic disease
- Activated circulating platelets have been identified in:
  - Unstable Angina
  - Acute Myocardial Infarction
  - Coronary Artery Disease
  - PCTA
- Platelet-Leukocyte aggregates have also been identified in Cardiac Disease
- In order to assess the true activation state, artifactual activation due to specimen handling, age, anticoagulant, etc. must be excluded
- Differences in platelet response to agonists may be useful in evaluating platelet function

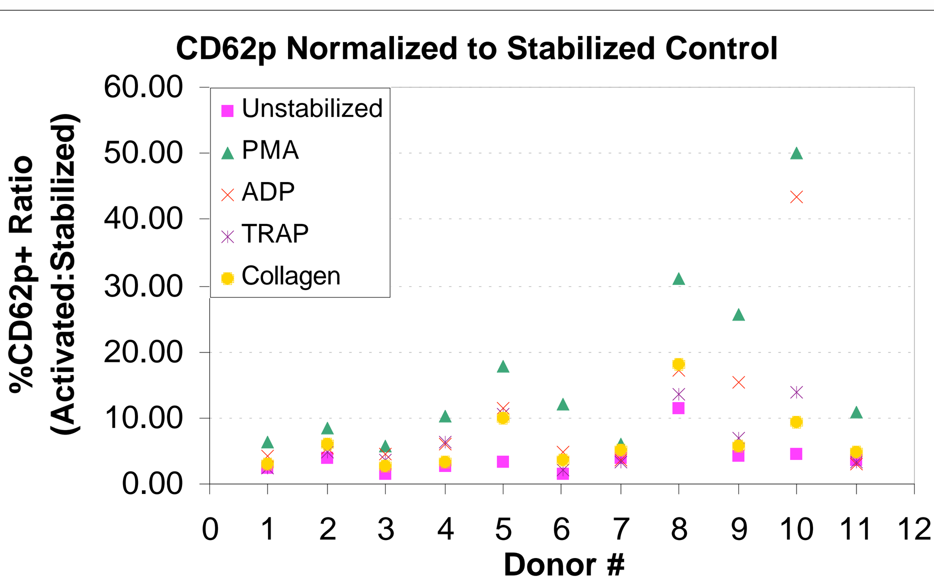
Materials & Methods

- 3 - 10 µL of a platelet agonist (PMA, ADP, TRAP, Collagen) was added to 200 µL citrated whole blood and incubated for 1.5 hours. Another aliquot of blood was left untreated for this time.
- 5.5 µL activated blood or untreated blood, or 11 µL whole blood pre-stabilized with ThromboFix™ was added to a cocktail of target cellular markers including CD14-FITC or CD31-FITC, CD62p-PE, CD45-ECD, and CD41-PC7 (Beckman Coulter, Inc.). CD142 (Sunol Molecular Corporation) was conjugated to PC5 for use in this assay.
- The samples were incubated for 10 minutes at 25C.
- The samples were then diluted with 1 mL PBS and analyzed on a Cytomics FC500 flow cytometer.

%CD62p and Ratio to Stabilized Control (ThromboFix-treated)

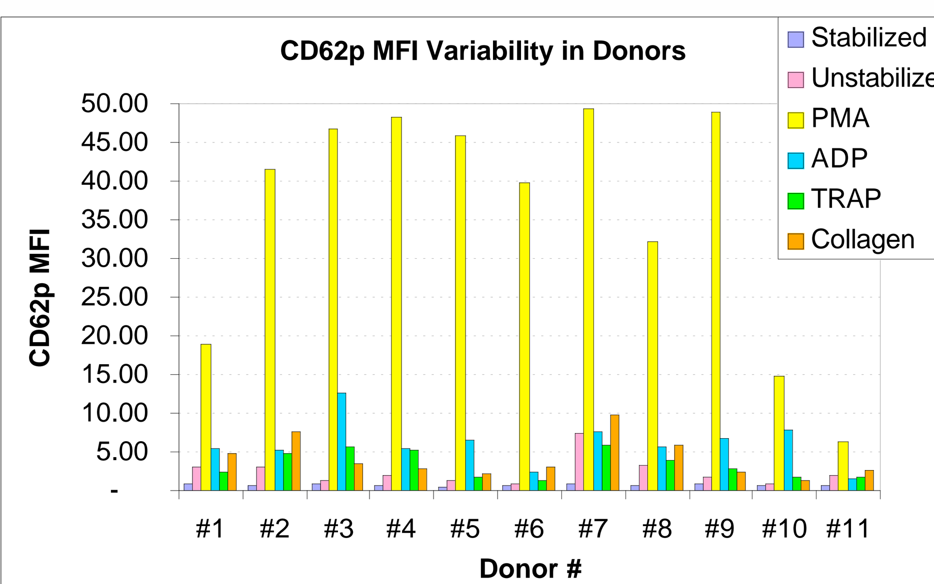
- PMA produced a relatively uniform response in terms of %CD62p at the dose tested; however, significant cell loss occurred
- More variability among donors was apparent with ADP, TRAP, and Collagen
- When the percentages observed were normalized to the stabilized control, the greatest difference among donors was produced by PMA and ADP
- The normalized values appeared to provide the clearest indication of donor variability

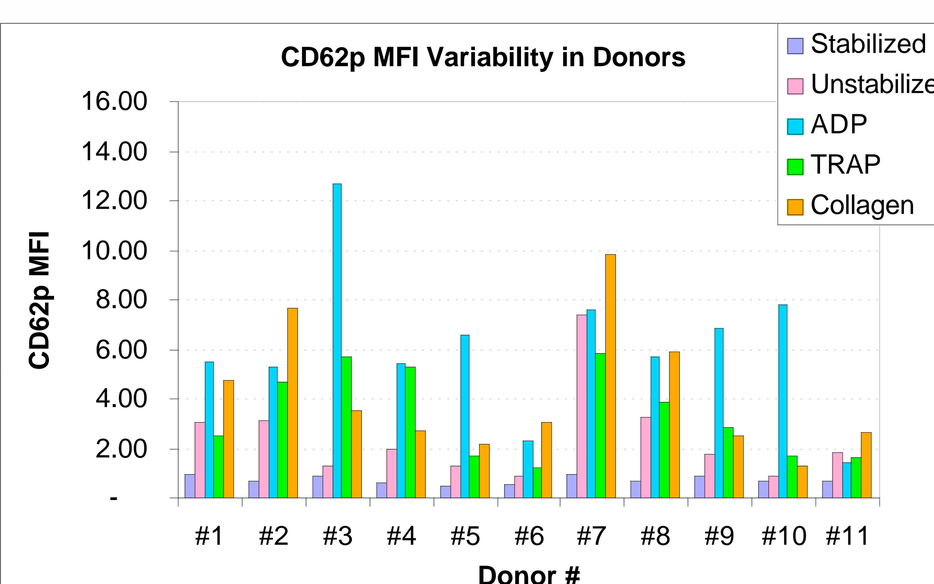




CD62p Fluorescence Intensity

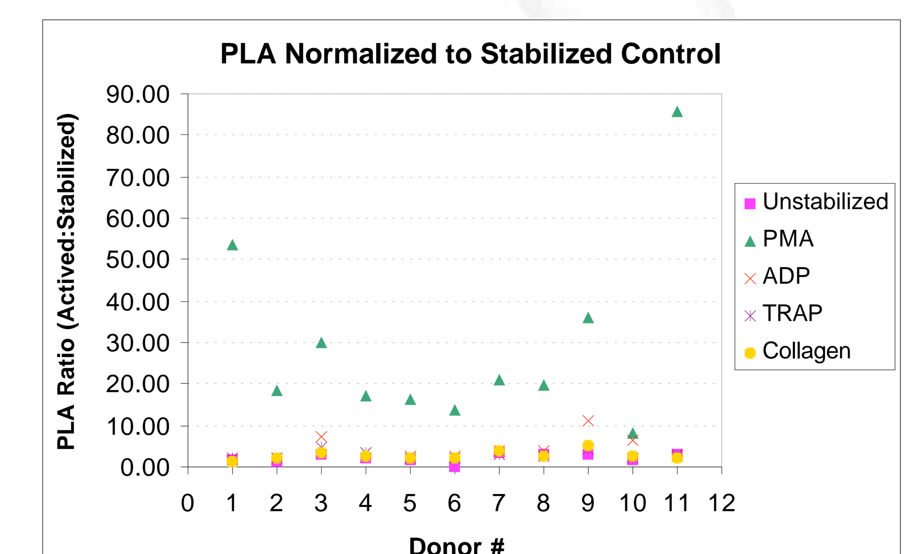
- Donor-dependent variations in the CD62p mean fluorescence intensity were observed for all agonists used as well as in untreated specimens
- ThromboFix treated specimens maintained a low, consistent fluorescence intensity

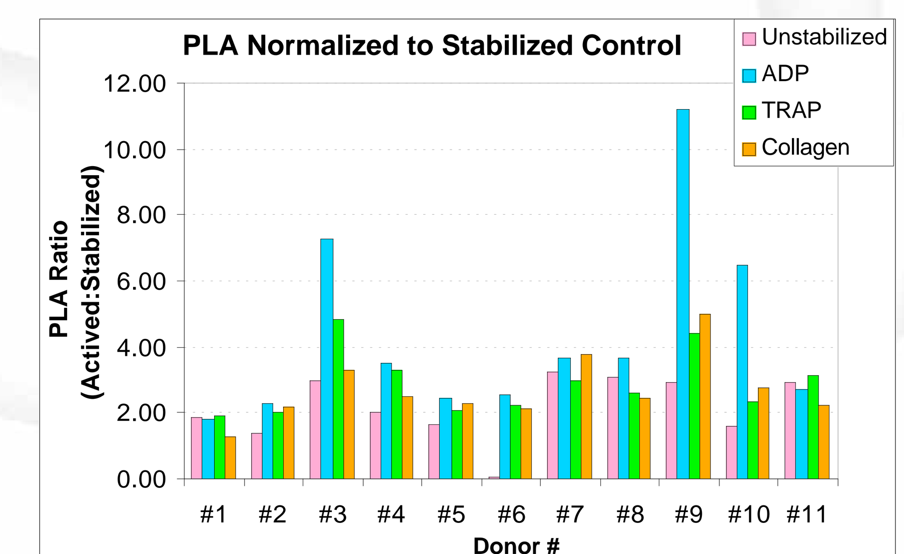




Platelet-Leukocyte Aggregates (PLA)

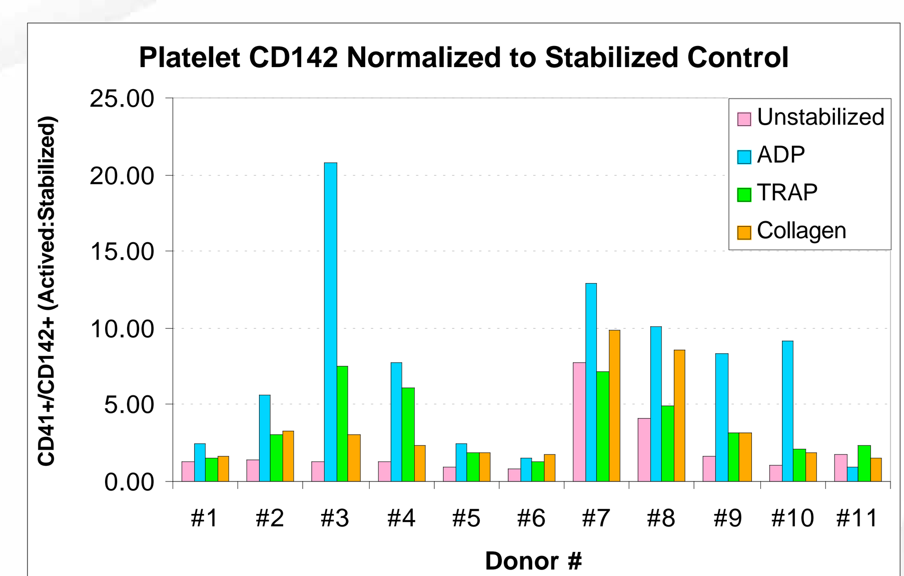
- The highest variation among donors was observed with PMA treatment
- ADP challenge also resulted in aggregate formation that was discernable from unstabilized blood





Tissue Factor (CD142)

- On platelets, expression of CD142 was most evident in response to activation by PMA with much smaller variations observed with ADP
- CD45+/CD14+ leukocytes generally expressed more CD142 in response to PMA challenge; however, differences among the agonists were small



Conclusions/Discussion

- Use of ThromboFix to stabilize platelets provides a standardized reference for the systematic evaluation of platelet reactivity
- Differences in individual response to physiological agonists were observed with respect to P-selectin (CD62p), Platelet-Leukocyte Aggregates, and platelet-associated Tissue Factor
- The platelet activation potential may provide a useful tool for the investigation of cardiac disease