Platelet Activation in Stored Platelet Concentrates: Comparision of Two Methods Preparation

Soleimany Ferizhandy Ali

Iranian blood transfusion organization-research center, hemmat Exp way, P.O.Box:14665-1157, Tehran, Iran

Abstract

Background and Objectives: This study has determined in vitro quality of 5 days stored platelet concentrates prepared by two different methods. Preparation conditions of platelet may cause platelet activation, which contributes to decreased ability of stored platelet to function. The quality platelets concentrate plays an important role in transfusion therapy. Their quality was assessed using the following parameters: platelets, leukocytes and erythrocytes counts, pH, P-selectin (CD62P) and Annexin V. P-selectin and Annexin V can be detected on the activated platelet. Annexin V was used as a parameter for quality monitoring of platelet concentrates during storage. The present paper compares quality properties of both platelet preparations in vitro.

Methods and Materials: In this experimental study, 30 platelet concentrates prepared with platelet rich plasma-platelet concentrates and 30 units via buffy coat-derived platelet concentrate methods. The percentages of Annexin V, P-selectin expression, platelet, leukocytes and erythrocytes counts and pH were evaluated.

Results: During storage for up to 5 days, buffy coat-derived platelet concentrates units displayed no significant pH difference in comparison with platelet rich plasma-platelet concentrates preparation (p>0.05). The mean leukocytes count buffy coat-derived platelet concentrates and platelet rich plasma-platelet concentrates was comparable and statistically significant difference was observed (p<0.05). During storage for up to 5 days platelet rich plasma-platelet concentrates units displayed significant an increase in the CD62P, Annexin V expressions, as compared with buffy coat-derived platelet concentrates preparation on day 5 (p<0.05).

Conclusions: The kinetics of CD62P and annexin V levels are influenced by the method used to prepare platelets for storage. The different levels of CD62P and annexin V in buffy coat-derived platelet concentrates and platelet rich plasma-platelet concentrates clearly demonstrating a progressive activation process of platelet rich plasma-platelet concentrates exceeds that of buffy coat-derived platelet concentrates.

Keywords: P-selectin; Annexin V; Buffy coat

Introduction

Preparation conditions and storage of platelet for transfusion may cause platelet activation, which contributes to decreased ability of stored platelet to function and to survive in vivo after transfusion compared with that seen with freshly prepared platelets [1-4]. Platelet transfusion therapy has played an important role in the management of patients [2,4]. Today, platelet concentrates are routinely manufactured from whole blood by differential centrifugation buffy coat-derived platelet concentrates (BC) or by platelet rich plasma-platelet concentrates (PRP-PC) and platelepheresis [5-7]. Whereas in 1970 and 1985, the use of BC exceeded that of PRP-PC by far. We here report on the difference between both platelet concentrates in functional properties of platelet in BC and PRP-PC are compared over storage of 5 days. Processing and storage may affect both platelet morphology and function and they may thus be less effective when transfused than fresh platelets [1,3-4,8-9]. There are several methods for quality control of platelet components including cell counting, pH, swirling and markers for platelet activation [8,10-11]. Redistribution of phosphatidylserine (PS) from the inner membrane surface to the exterior surface accompanies during platelet activation [11,12,13]. The exposure of PS can be measured by using flow cytometry and fluorescent-labeled annexin V. Annexin V was used as a parameter for quality monitoring of platelet concentrates during storage. This essay is based on the principle that annexin V binds to PS, with high affinity and high specificity [8,12-14]. Activation of thrombocytes is followed by an increased CD62P expression; this protein is the main component of the alpha granules secreted from the granules during storage [7-8]. The aim of this study is to characterize the quality of the changes occurred in the platelet activation parameters during the storage of platelet concentrates derived from BCs and PRP-PCs.

Materials and Methods

Platelet preparation

Preparation of buffy coat - platelet concentrate (BC): Thirty whole blood was collected in a 450-ml quadruple bag containing 63 ml of CPDA1 anticoagulant. (TERUMO PENPOL,Ltd., Puliyarakonam, Trivandrum, India). The whole blood was first subjected to "hard spin" centrifugation at 3840 rpm for 6 minutes at 22°C with acceleration and deceleration curves of 7 and 3 respectively. Whole blood was separated into different components according to their specific gravity. After centrifugation the supernatant plasma and the subnanat red cells were transferred into attached satellite containers. Platelet poor supernatant was expressed into one satellite bag and buffy coat into another satellite bag. About 20-30 ml of plasma was returned to buffy coat with the aim of cleaning the tubing from residual cells and obtaining an appropriate quality of 5 days stored platelet concentrates in vitro.
**Table 1:** Cell counts and pH in buffy coat-derived platelet concentrate (BC) and platelet rich plasma-platelet concentrates (PRP-PC).

<table>
<thead>
<tr>
<th>PRP-PC</th>
<th>BC</th>
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<tbody>
<tr>
<td><strong>Days</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>Platelet/unit x 10^10</strong></td>
<td>5.8±1.1</td>
</tr>
<tr>
<td><strong>WBC/unit x 10^6</strong></td>
<td>44±0.65</td>
</tr>
<tr>
<td><strong>RBC/unit x 10^7</strong></td>
<td>27±2.2</td>
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<tr>
<td><strong>pH</strong></td>
<td>6.9±0.12</td>
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</table>

Values are mean± standard deviation; n=30

**Table 2:** Platelet activation markers are stored in buffy coat-derived platelet concentrate (BC) and platelet rich plasma-platelet concentrates (PRP-PC).

<table>
<thead>
<tr>
<th>PRP-PC</th>
<th>BC</th>
</tr>
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<tbody>
<tr>
<td><strong>Days</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>CD62P %</strong></td>
<td>13.4±9.7</td>
</tr>
<tr>
<td><strong>Annexin V %</strong></td>
<td>3.3±0.9</td>
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</table>

Values are mean± standard deviation; n=30

Discussion

This study has determined in vitro quality of 5 days stored platelet concentrates prepared by two different methods. The detection of platelet activation has been used as a guide to the assessment of the suitability of techniques or conditions under which platelets have been prepared for transfusion. The present paper describes an in vitro comparison of both preparations. The studies suggested that the degree of in vitro activation as evidenced by the expression of CD62P and Annexin V binding were dependent on the different preparative methods [4-16,17,18]. We found that the extent of activation was significantly higher in PRP-PCs than in BCs. It was concluded that, immediately after preparation, PRP-derived platelets are more activated than BC-derived platelets. This is most likely a result of the pelleting that follows the second high-speed centrifugation of the PRP. The results show that Annexin V binding and CD62P increase after preparation of platelet concentrates from PRP-PCs and BC, and rises progressively during storage under blood bank conditions. Although in both preparations, platelet activation is increased by storage time, BCs are characterized by a much better in vitro than PRP-PCs [3-5,7]. However, the different CD62P and Annexin V in PRP-PCs and BCs clearly demonstrate that process of activation exceeds that of BCs. Platelets processed by the buffy coat technique showed less p-selectin (CD62P) expression than platelets prepared by the PRP method.
study shows that in addition to pH and cell counts, in vitro platelet activation markers were used to monitor platelet quality [11,19]. These data suggest that measurement of CD62P and Annexin V may be a more desirable marker for clinical studies of activated platelets, since it may be less susceptible to artifactual elevation due to minor variations in sample handling and assay procedures [13-15].

Acknowledgement

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References