Transfusion and Apheresis Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Superior integrin activating capacity and higher adhesion to fibrinogen matrix in buffy coat-derived platelet concentrates (PCs) compared to PRP-PCs

Pezhman Beshkar[a], Ehteramolsadat Hosseini[a], Mehran Ghasemzadeh[a,b,*]

[a] Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
[b] Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria 3004, Australia

ARTICLE INFO

Article history:
Received 9 October 2017
Received in revised form
12 December 2017
Accepted 12 December 2017
Available online xxx

Keywords:
Buffy coat (BC)
Fibrinogen
Integrin alpha IIb beta 3 ($\alpha_{IIb}\beta_3$)
Platelet adhesion
Platelet-rich plasma (PRP)

ABSTRACT

Background: Regardless of different sources, methods or devices which are applied for preparation of therapeutic platelets, these products are generally isolated from whole blood by the sedimentation techniques which are based on PRP or buffy coat (BC) separation. As a general fact, platelet preparation and storage are also associated with some deleterious changes that known as platelet storage lesion (PSL). Although these alternations in platelet functional activity are aggravated during storage, whether technical issues within preparation can affect integrin activation and platelet adhesion to fibrinogen were investigated in this study.

Methods: PRP- and BC-platelet concentrates (PCs) were subjected to flowcytometry analysis to examine the expression of platelet activation marker, P-selectin as well as active confirmation of the GPIIb/IIIa ($\alpha_{IIb}\beta_3$) on day 0, 1, 3 and 5 post-storage. Platelet adhesion to fibrinogen matrix was evaluated by fluorescence microscopy. Glucose concentration and LDH activity were also measured by colorimetric methods.

Results: The increasing P-selectin expression during storage was in a reverse correlation with PAC-1 binding ($r = -0.67$; $p = .001$). PRP-PCs showed the higher level of P-selectin expression than BC-PCs, whereas the levels of PAC-1 binding and platelet adhesion to fibrinogen matrix were significantly lower in PRP-PCs. Higher levels of active confirmation of the GPIIb/IIIa in BC-PCs were also associated with greater concentration of glucose in these products.

Conclusion: We demonstrated the superior capacities of integrin activation and adhesion to fibrinogen for BC-PCs compared to those of PRP-PCs. These findings may provide more advantages for BC method of platelet preparation.

1. Introduction

Despite the long experience in the production of platelet concentrates (PCs) for transfusion purposes, the preparation and storage of these products still remain as an important challenge for the blood transfusion services [1]. This problem is often raised by the special condition of platelet storage at 20–24 °C, which makes these products susceptible to either metabolic stress or bacterial contamination. Storage-dependent morphologic and metabolic changes can limit the quality and shelf life of PCs while inducing some reversible and irreversible effects on the platelet functional activities. These are deleterious changes that known as platelet storage lesion (PSL). The PSL comprises a group of defects that gradually affect the structure and function of the platelets. At the earlier stage, these changes begin with some reversible cytoskeletal and metabolic abnormalities which then develop to surface membrane antigen and ligand integrity due to irreversible granule release and receptor/microparticle shedding. The abnormalities eventually lead to platelet pro-coagulant function and apoptosis which tremendously affect platelet quality [2]. So far, several lines of evidence showed that different techniques in platelet preparation can also affect platelet quality and functional activities [3–5]. Generally, PCs are produced by either platelet-rich plasma (PRP) or buffy coat (BC) which is obtained from whole blood donated by random donor

* Corresponding author. Present address: Blood Transfusion Research Centre, High Institute for Research and Education in Transfusion Medicine, Iranian Blood Transfusion Organization Building, Hemmat Exp. Way, Next to the Milad Tower, PO Box 14665-1157, Tehran, Iran.
E-mail addresses: m.ghasemzadeh@ibto.ir, mehran1476@yahoo.com (M. Ghasemzadeh).

https://doi.org/10.1016/j.transci.2017.12.003
1473-0502/

or collected from specified single donor during platelet apheresis. While in some countries buffy coat technique is more commonly used, in the majority of developing countries PRP platelets are still considered as the only protocol for platelet preparation from blood donated by random donor [6].

Studies have shown that the second stage of centrifugation in the PRP method can cause reversible platelet aggregation due to the intimate contact between platelets .The possible release of platelet agonist during this cross –contact may also induce platelet activation at the later stage of storage. On the other hand, in buffy coat-based platelet preparation, technically having less platelet-platelet contact during centrifugation may protect platelets from pre-activation [7].

The observation of higher levels of granule release and platelet aggregation in PRP platelet than BC-originated one can support obvious engagement of inside-out signaling pathways in PRP platelets [8–10]. It seems that in the later stage of platelet storage, the higher levels of these preliminary signals also affect the activation state of platelet integrin, $\alpha_{IIb}\beta_3$ as a prerequisite for further platelet activation [11]. However, whether both platelet release and integrin activation state could be differently affected by platelet preparation technique during storage was of interest in this study in which for the first time the platelet adhesion to fibrinogen matrix in addition to the expression of activated $\alpha_{IIb}\beta_3$ were compared between BC and PRP derived PCs.

2. Materials and methods

2.1. Reagents

Mouse IgG1, κ Isotype controls (PE, FITC and PerCP conjugated) were from Miltenyi Biotec (Germany). Monoclonal antibodies against human, CD42b (FITC or PE conjugated), CD62P (FITC or PerCP conjugated) and PAC-1 (activation-specific anti-GPIIb/IIIa antibody FITC cojugated) were from BD Pharmingen (USA). Other reagents and chemicals were from Sigma Aldrich (USA).

2.2. Sample preparation

The present study was conducted on the samples of 10 blood donors. The study was approved by the local ethical committee, and the informed consent was obtained from the blood candidates by Iranian Blood Transfusion Organization (IBTO). 5 PRP-PCs and 5 BC-PCs were prepared from whole bloods donated by volunteers. While PRP-PCs were prepared by IBTO's standard protocol, for BC-PCs preparation, the WB was collected in quadruple bags (top and Bottom) (Fresenius KABI,GmbH, Germany) including anticoagulant CPD and solution of saline, adenine, glucose and manitol (SAGM).WB was kept at room temperature (20–24 °C) overnight. The WB was then subjected to "hard spin centrifugation" at 4200 g for 14 min at 22 °C and then separated with automatic CompoMat G5 separators (Fresenius Kabi Italia, Verona, Italy). The SAGM solution was then added to the red cells and the bags containing red cells and plasma were removed. The buffy coat was gently mixed with 40–60 mL of plasma and kept at room temperature (20–24 °C) for 3 hours and then subjected to "light spin centrifugation" at 430 g for 10 min at 22 °C. The supernatant PRP was expressed into a PLT storage bag while the tubing was sealed. The bag with residual WBCs and red cells was then discarded (see Supplementary Fig. S1 in the online version at DOI:10.1016/j.transci.2017.12.003).

All PCs passed release process under IBTO screening regulations. Each bag included 50–60 mL PC with more than 1×10^9 PLT/mL. Under sterile condition, 5 mL of product was taken from the cord for the base-line study on day 0 of storage and after sealing back, the PCs were kept in a shaker incubator at 20–24 °C. At each time points (day 1, day 3 and day 5) and under sterile condition, samples were taken from each BC- or PRP-PC *via* the cord. For this purpose, after returning cord content to the original bag and mixing the bag, the cord was opened while a small amount of platelet (~1 mL) was first discarded, then 4 mL of the sample was collected in a sterile falcon tube. At the end, the cord was sealed a few centimeters closer to the bag which then returned to the shaker incubator at 20–24 °C for future use.

For each sample adding a designated amount of Tyrode buffer (10 mM Hepes, 12 mM $NaHCO_3$, 137 mM NaCl, 2.7 mM KCl, 5 mM glucose, 1 mM $CaCl_2$; pH = 7.4), platelets were washed and isolated as described previously [12]. For flowcytometry and adhesion analysis, platelet counts were adjusted to 2 × 10^7/mL.

2.3. QC parameters of PCs

Assessment of total volume, PLT count and WBC count were carried out on PCs after preparation and storage at each time points. PLT count (per unit) and WBC count (per unit) were measured using a hematology full blood analyzer (XE-2100, Sysmex,Milton Keynes, UK).

2.4. Platelet Metabolic analysis during storage

Measurement of pH was performed at 22 °C on a pH meter (826 pH mobile/827 pH lab, Metrohm AG, Switzerland) for stored PCs at each time points. Glucose and LDH activity were also measured by colorimetric methods (kits obtained from Randox, UK) using a Chemistry Analyzer (Hitachi 911, Japan). For glucose, the samples were incubated in the appropriate reaction mixture for 10 min at room temperature, and the absorbance was measured at 570 nm, while LDH activity was evaluated at 340 nm with pyruvate-lactate method. Standard curves were applied to assess the values of these parameters.

2.5. Bacterial cultures

Bacterial cultures were performed on day 1 and 5 for each bag of platelets, including both aerobic and anaerobic cultures.

2.6. Flowcytometry analysis

Platelets were stained with either anti P-selectin (CD62P), GPIbα (CD42b) or anti PAC-1 (against activation-site of GPIIb/IIIa). After incubation time, cells were then fixed in 1% paraformaldehyde in PBS and subjected to flowcytometer (CyFlow® *Space, Partec* GmbH, *Germany*) where a total of 20,000 PLT events were acquired. The flowcytometer settings were optimized for the acquisition of platelets by logarithmic signal amplification in all four detectors (forward and side scatter channels and fluorescence channels FL1/FL2). For analysis, the gate was set around intact PLT population as defined by forward and side scatter characteristics and confirmed by the presence of platelets expressing GPIbα. The percentage of positive platelets expressing P-selectin above the background (negative control) was recorded while PAC-1 binding quantified as the mean fluorescence intensity (MFI). Data were analyzed with FLOWJO software (Tree Star Inc, OR, USA).

2.7. Static platelet adhesion

Glass coverslips (12 mm in diameter) were coated with fibrinogen (100 μg/mL) for 2 hours at room temperature or overnight at 4 °C. Unbound proteins were removed by washing the coverslips three times with Tyrode's buffer and the uncoated glass surface was blocked with 2% human serum for 30 min at room temperature. Excess blocking solution was removed by three

Please cite this article in press as: Beshkar P, et al. Superior integrin activating capacity and higher adhesion to fibrinogen matrix in buffy coat-derived platelet concentrates (PCs) compared to PRP-PCs. Transfus Apheresis Sci (2017), https://doi.org/10.1016/j.transci.2017.12.003