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Flow Cytometric Measurement of CD41/CD61 and CD42b Platelet Receptors and Clotting Assay of Platelet Factor 3 During Long Term-Storage of Platelet Concentrates

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Abstract

Background: The purpose of the present in vitro study was to evaluate the effect of long term storage of conventional platelet concentrates (PCs) on major platelet receptors CD42b and CD41/CD61 by flow cytometry method and also measuring the overall platelet procoagulant activity status using platelet factor 3 (PF3) assay.

Materials and Methods: Six random units of conventional platelet concentrate were prepared and after collection and resting period, were placed in a standard platelet incubator under continuous agitation at 22-24°C for eleven days. Samples of each platelet concentrate unit were taken on days 1, 5 and 11 after their preparation. Two types of samples; one pooled sample for flow cytometry analysis and also individual samples for PF3 assay were prepared and tested.

Results: Flow cytometric analysis of CD41, CD61 and CD42b found 99.8%, 94.8% and 28% activity on the first day which decreased to 68.3%, 75.1% and 3.4% respectively at the end of the storage period (11 days). The overall platelet procoagulant activity status measured by PF3 assay was 95.0% and 41.7% at the beginning and the end of the storage period respectively.

Conclusion: Our results indicated that storage lesion occurs during long-term storage of platelets with a nearly constant slope. In spite of lower expression of CD42b, higher expression levels of CD41/CD61 and platelet procoagulant activity measured by PF3 method were observed at the end of the storage period which confirms that platelet procoagulant properties are moderately preserved even after an eleven days period.

Keywords: CD41/CD61, CD42, PF3, platelet concentrate, flow cytometry.

Introduction

Human platelets (PLTs) are anucleate, discoid small cells (2–4 μ by 0.5 μ) that normally circulate at concentrations of 150 - 400 × 109 /L , higher in women than in men despite lower thrombopoietin levels [1], for a maximum of 10 days. They are primed to undergo explosive liberation/activation following damage from the vessel wall. This leads to the rapid formation of a vascular plug to occlude the site of damage. PLTs are therefore enriched in signaling proteins and surface glycoprotein receptors (GP) that enable them to sufficiently respond to vessel wall injury [2]. Since Murphy and Gardner [3] reported that platelet concentrates (PCs) were best preserved at 22° C rather than 4°C, there has been little improvement in how PCs are stored. Currently, platelet concentrates are stored at 22° C for only five days because prolonged storage has been associated with bacterial proliferation and transfusion associated septicemia [4,5]. It is suggested that even if bacterial growth could be avoided, PCs would have to be transfused by the seventh day of storage at 22°C due to activation and loss of platelet function [6].

The GPIb-IX-V complex is a membrane receptor complex on the surface of platelets that mediates the first critical step in platelet adhesion by

facilitating binding to vWF on damaged sub-endothelium under conditions of high fluid shear stress [7-9]. Aggregation begins after activation, and occurs as a result of turning on of the GPIIb/IIIa receptor, which allows these receptors to bind with vWF or fibrinogen [10]. There are ~80000 molecules expressed per platelet [11]. When any one or more of at least nine different platelet surface receptors are turned on during activation, intra-platelet signaling pathways cause existing GpIIb/IIIa receptors to change from curled to straight form and thus become capable of binding [10].

Platelet factor 3 as a phospholipid lipoprotein blood coagulation factor derived from platelets acts with certain plasma thromboplastin factors to convert prothrombin to thrombin. The PF3 assay relies on the principle that incubation of platelet-rich plasma (PRP) with kaolin activates the procoagulant activity of platelets, resulting in a progressive shortening of both the recalcification time and Russell viper venom time. By appropriate mixing of normal platelet-poor plasma (PPP) and PRP from a patient, the PF3 test can be made specific for assessing the platelet procoagulant activity (PCA) [12-15].

The purpose of the present in vitro study was to evaluate the effects of long term storage of conventional platelet concentrates (PCs) on major adhesion and aggregation parameters of platelet receptors CD42b and CD41/CD61 by flow cytometry method as well as measuring the overall platelet procoagulant activity status using PF3 method.

Materials and Methods

Six random units of conventional platelet concentrate were prepared in Tehran Blood Transfusion Center as following a routine procedure. After collection, standard PCs were kept undisturbed for one hour, then, they were placed in a standard platelet incubator with shaker (Danesh Pajoohesh Fajr Co., Iran) under continuous agitation at 22-24°C for eleven days. Three milliliter samples of each PC unit were taken on 1, 5 and 11 days after their preparation. Two types of samples; one pooled sample for flow cytometry analysis as well as individual samples for PF3 assay were prepared.

Flow cytometry analysis

The polled sample was tested using a CyFlow® ML flow cytometer (Partec GmbH Otto-Hahn-Straβe 32, D-48161 Munster, Germany) equipped with a 488-nm argon-ion laser and 635-nm red-diode laser. For analysis of platelets in washed platelet suspensions, fluorescence parameters and light scattering were adjusted at logarithmic gain, and the threshold was set to eliminate events negative for platelet markers. Quality control procedures were followed; the flow cytometer setting was identical for all analyses and negative and positive controls were performed routinely. Single platelets were identified by their characteristic forward light scatter and high CD42b fluorescence.

PF3 assay

Platelet factor 3 assay was performed on six individual samples on days 1, 5 and 11 of platelet concentrates storage according to the kaolin clotting time method 12. Fresh platelets and physiologic normal saline were used as positive and negative controls respectively. The clotting time was converted to percent activity by use of a standard curve.

Results

Our results presented in Table 1 showed that the expression of CD41/CD61 can be retained to some

Table 1: Flow cytometry analysis of the pooled* sample of platelet concentrates stored for eleven days.

Assay (%)	Day 1 activity (%)	Day 5 activity (%)	Day 11 activity (%)
CD42b	28.0	13.9	3.4
CD41	99.8	82.4	68.3
CD61	94.8	80.6	75.1

*Six conventional platelet concentrates were stored for eleven days and the test was performed on the pooled sample.

Table 2: The results of Platelet factor 3 (PF3) assay on six platelet concentrates stored for eleven days.

Assay	Day 1	Day 5	Day 11
PF3 (sec)	9.6±3.2*	19.3±2.9	30.8±1.9
PF3 (%)**	95.0±5.8	71.4±5.2	41.7±3.4

*µ±2SD
** Percent activity was calculated by use of a standard curve.

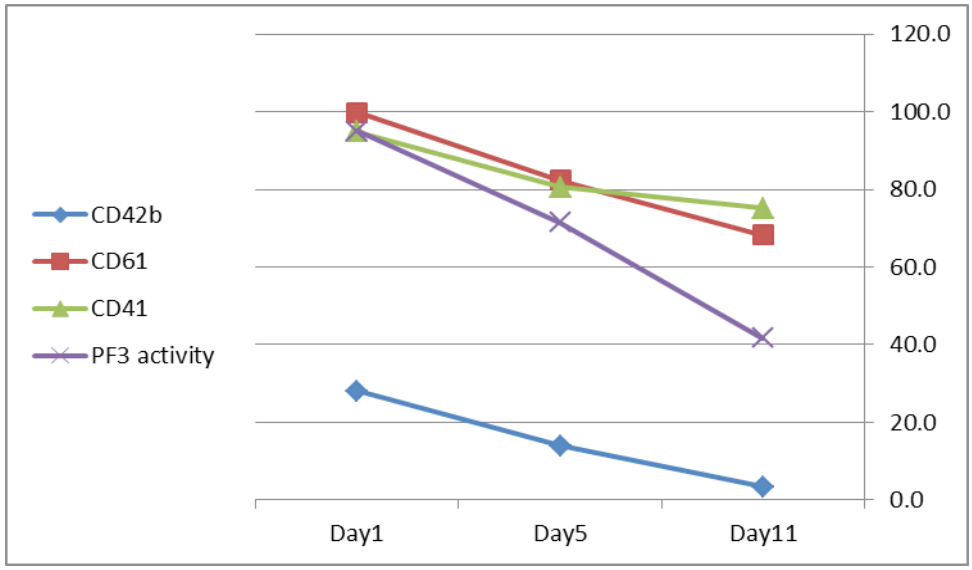

Figure 1: Flow cytometry measurement of CD42b, CD41/CD61 receptors as well as the results of PF3 clotting activity assay during storage of platelet concentrates for eleven days.

degree even eleven days after platelet preparation with 68.3% and 75.1% activity respectively, but much lower expression of CD42b was seen in the pooled samples at the end of study. The expression of these three receptors constantly declined during the long-term standard storage due to platelet storage lesion (PSL) process in platelet concentrates (Figure 1).

Platelet procoagulant activity in six platelet concentrates was measured using PF3 method during 11 days storage showing 41.7% activity at the end of the storage (Table 2, Figure 1.)

Discussion

Generally, platelet in vitro function declines due to a collection of biochemical, morphological and functional changes that occur to platelets during the storage period; the so-called platelet storage lesion[16,17]. In agreement with previous data, our results showed that there was moderate reduction in the expression of CD41/CD61 (αIIb/ßIII), a subunit of fibrinogen receptor glycoprotein during storage [18-20]. CD42b is a subunit of the GPIb-IX-V complex, and is the receptor for vWF and a high affinity receptor for thrombin [21]. The expression of this receptor in our study was lower in comparison with other studies which may be due to the more

shedding of these receptors during storage [22] and therefore, needs to be further investigated for soluble CD42b determination in the platelet concentrates during storage in the future. On the other hand, previous studies have shown that the stimulation-induced redistribution of VWF receptor (GPIb/IX complex) on the platelet surface membrane is reversible. In short, thrombin induced myosin II stimulation and actin assemblies cause GPIba/IX complex on the resting surface membrane to translocate to the intraplatelet pool, resulting in a rapid decrease in the complex on the platelet surface membrane, and then a reappearance of the same molecules to the surface membrane, but their physiological role remains unclear [23-25]. In this relation, it is noteworthy that poor expression of CD42b might result in reduced platelet function; however, blockade of 50% of CD42b sites on platelets does not affect platelet adhesion under flow conditions in vitro [26].

In our results, PF3 as a major procoagulant activity test of platelets retained its relative activity with 41.7% activity even after eleven days. This confirms that these outdated platelets still have sufficient procoagulant activity even after 11 days. In this test, phospholipid (as a partial thromboplastin) and ionic calcium, as well as an activator of the contact factors (Kaolin) at the presence of normal plasma (including coagulation factors, prothrombin, fibrinogen,..) were used to activate coagulation pathways. PF3 test can show the overall procoagulant activity of platelets by activating coagulation factors and may incorporate other receptors such as CD41/CD61 for fibrinogen attachment in platelet aggregation. This procoagulant activity of platelet membrane may be used as a platelet substitute in form of infusible platelet membrane in the future [27-31].

Platelet storage lesion can affect quality of platelet concentrates during storage and may affect platelet ultrastructure, function, metabolism, and membrane properties, but the process of platelet preparation and appropriate additive solutions may improve its quality during storage [32,33].

Conclusion

Our results indicated that storage lesion occurs during long-term storage of platelets with a nearly constant slope. In spite of lower expression of CD42b, higher expression levels of CD41/CD61 and platelet procoagulant activity measured by PF3 method were observed at the end of the storage period which confirms that platelet procoagulant properties are moderately preserved even after an eleven days period.

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