

The Effect of (Non-) Agitating Condition on Agonist Induced-Aggregation of the 48 hours-Stored Platelet Concentrates

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Abstract

Platelets (PLTs) transfusion and their efficacy in a recipient depend on PLTs quality and quantity pretransfusion. One of the important aspects of treating PLT concentrates (PCs), which has controversial effect on functionality is storage of the PCs in an active metabolic state during prolonged storage either by (gentle-) agitation or by rotations in the different angels.

Study design and Methods: The amount of PCs obtained through double centrifugation methods, as described. We measured ADP, Collagen and ristocetin-induced aggregation after 48 hours of storage in PC kept under continuous agitation (CA6h), and in those in which agitation was stopped in the last 6 hours i.e Without Continuous Agitation for 6 Hours (WCA6h).

Results: In Timori *et al.* BT 201 we have shown that an interruption of 6 hours on PCs has no deleterious effect on PCs P-selectin, PF4, LDH release, pH, swirling, and count. In this study we focus more in details about different agonist induced-aggregation functions, and their receptors' (ir-) responsiveness and (de-)sensitivity, signal transduction pathways, of the WCA6h and CA6h stored counterparts. The mean level of aggregation response to collagen, ADP and ristocetin in agitated CA6h versus WCA6hr were $3.49 \pm 1.73\%$ vs. $3.46 \pm 1.0\%$ ($p < 0.962$), $4.30 \pm 2.7\%$ vs. $3.20 \pm 3.9\%$ ($p < 0.518$), and $79.2 \pm 4.4\%$ vs. $66.65 \pm 28.55\%$ ($p < 0.186$), respectively.

Discussion: We observed no significant differences between different receptors affected by interruption to respond for firm aggregates. Thus, a short period of 6 hours non-agitating condition after 42 hours continue agitation in the permeable bags had not deleterious effect on PCs agonist-induced aggregation. Compared to disrupted, continue agitation has no superior effect on old PCs' (ir-) responsiveness and (de-)sensitivity after 48 hours.

Keywords: Platelets; Storage; Blood banking Agitation; Metabolism; Receptors; ADP; Collagen; Ristocetin; GPIb-V-IX complex; Signal transduction; Aggregation

Introduction

Platelets (PLTs) transfusion and their efficacy in a recipient depend on PLTs quality and quantity pretransfusion. Quality control of platelet concentrates (PCs) stored for more than 3 days is not so easy task during prolonged storage and transportation (Figure 1). However, due to PLT membrane lesions it is complicated to test all PLTs feature in the Blood banking Centers (BCCs) [1]. Though, agonist-induced three different functions of PLTs namely activation, adhesion, aggregation (triple A's) (in an ideal world) should be tested either pre- or posttransfusion. Such investigations reveal whether certain PCs stored for more than 1 day are functional or not (Figure 2).

PLT's receptors (ir-) responsiveness, quality and quantity also have a significant impact on either the primary or secondary homeostasis, as well [2-4].

In recipients, (ir-) responsiveness, (de-) sensitivity of PLTs to small amounts of thrombin is crucial [5,6]. Different receptors hereby play a pivotal role i.e PAR1 and PAR4 are protease-activated receptors and are responsible for thrombin reactivity of PLTs to interact and initiate primary and secondary homeostasis processes [6]. Upon PLT storage, ADP and ADP-receptors are essential to ensure adequate formation of aggregates and thrombi, [5,7] collagen and collagen-receptors are involved in primary homeostasis and thrombosis [8,9]. The ristocetin-vWF and the GPIb-V-IX complex receptor are involved in not only

primary homeostasis but also in clearance of cold and old PLTs [10,11]. Any defect in these receptors integrity and signal transduction system results in severe bleedings disorders [12].

In an emergent system several important methodological questions raise about PLTs efficacy, and their corrected count increment (CCI), in vivo. One of the important aspects of treating platelet concentrates (PCs), which has controversial effect on PLTs functionality and viability is storage of the PCs in an active metabolic state during prolonged storage either by (gentle-) agitation or by rotations in the different angels at room temperature [13]. Agitation technology was developed to maintain pH of PCs in the permeable bags after the collecting and separating the fresh PLTs from whole bloods [13-15].

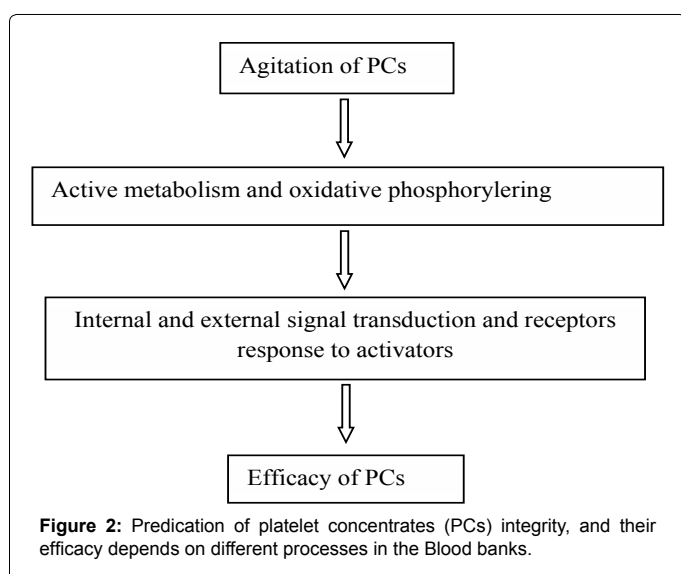
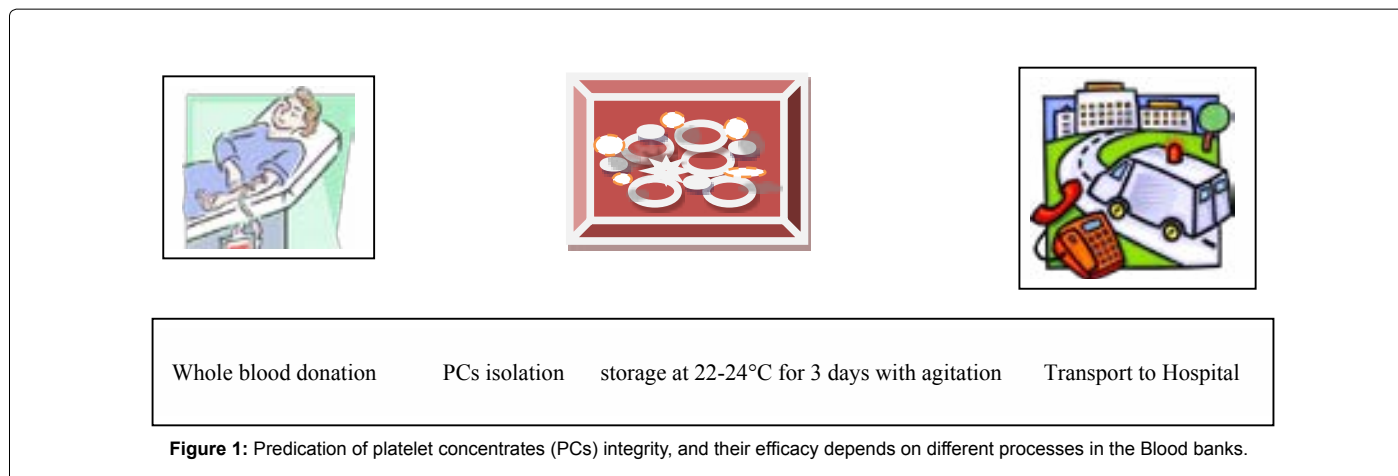
The PCs efficacy and functionality play pivotal role posttransfusion, as well. Different factors affect PCs quality and quantity [5,16,17]. How? And which factor(s) has/have direct relation to in- vivo PCs efficacy is not elucidated, completely. Predication of PCs efficacy and functionality is very important task in Transfusion Medicine and

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patients' safety. Hence we believe that study of different PLTs signaling, and their relevant receptors-response to various activating agents during aggregation is uttermost important process.

It is also obvious that other factors affect PCs efficacy in a recipient i.e. thrombolytic drugs for example Aspirin, Clopidogrel, Heparin, Warfarin and patients' genotype and phenotypes; which is out of focus of this paper.

The aim of this study is to investigate whether interruption of continue agitation for 6 hours as described [1]. has any further deleterious effect on PCs receptors' capabilities to respond to 3 different agonists (collagen, ADP and Ristocetin) to form firm aggregates in the blood bank of Tehran. Furthermore, is especially focused on the 3 different signaling pathways after interruption of agitation on activation, agglutination and aggregation, using 3 different agonists.

Here we report that 6 hours non agitating condition has no deleterious effect at different agonist-induced aggregation function of old PCs, pretransfusion. Compared to disrupted samples, continue agitation had no superior effect on agonist induced aggregation signaling.

Materials and Methods

PCs were prepared in the Iranian Blood Transfusion Organization

(IBTO) in Tehran, and stored for up to 48 hours in a standard shaker/incubator ($22 \pm 2^\circ\text{C}$) under continuous agitation. A cross-sectional study was conducted in the quality control department of the IBTO. In this study PLTs were collected from voluntary blood donors, who had signed informed consent forms, and whose donations were used to produce PCs (n=20). The PCs were obtained through methods, as described [1]. We measured ADP, collagen and ristocetin-induced aggregation after 48 hours of storage in the PCs kept under continuous agitation (CA6h), and in those in which agitation was stopped in the last 6 hours (WCA6h). The agonist-induced aggregations were measured by Aggregolink machine 4-channels (Kordia, USA) with 500 μl PCs after addition of ADP (20 μM), collagen (10 $\mu\text{g}/\text{ml}$), and ristocetin (1500 $\mu\text{g}/\text{ml}$) for 15 min at 37°C with stirring at 1,000 rpm.

Spontaneous agglutinations were visually inspected and tested. Activation properties were investigated by follow ups of aggregation curves integrities before and after agonist's additions.

As described [1]. in- vitro measurements of PCs quality were carried out just after completion of the resting period (designated as the control group, WCA6h), and the results were compared with those of PCs continuously agitated in the same day (designated as the control group, CA6h). The in vitro variables measured were swirling, different activators -induced aggregation responses to the agonists i.e ristocetin (GPIIb-related function), ADP (ADP receptors), collagen (collagen receptors).

Results

PLTs receptors (ir-) responsiveness, (de-)sensitivity to the different agonists, and their in-vivo efficacy in a recipient depend on PCs isolation procedures, storage condition, and storage time; beside original quality and quantity gained from donors whole blood.

In Timori et al. 2013 we have shown that an interruption of 6 hours on PCs that were stored at $22-24^\circ\text{C}$ in permeable plastic bags, with standard agitation system after 42 hours has no deleterious effect on PCs' P-selectin, PF4, LDH release, pH, swirling, count and aggregation function [1]. Furthermore, we studied prolongation of non-agitating time up to 24 hours [18] and we observed again no significant differences between controls and that extra 18 hours non agitating condition on the PCs function and viabilities.

It is generally accepted that prolonged storage beside agitation interruptions of old PCs, could affect PCs in-vivo receptors responses due to membrane lesions [19-21]. In this study we focus more in details

about 3 different agonist induced-aggregation functions, and their receptors' (ir-) responsiveness and (de-)sensitivity, signal transduction pathways, of the WCA6h and CA6h stored counterparts.

Compared to fresh PLTs, aggregation responses to collagen, ADP in all samples either in CA6h (agitated) or non-agitated (WCA6h) PCs were decreased i.e. $3.49 \pm 1.73\%$ vs. $3.46 \pm 1.0\%$ ($p < 0.962$), $4.30 \pm 2.7\%$ vs. $3.20 \pm 3.9\%$ ($p < 0.518$), respectively (Table 1). Recall in these responses is mainly GPIb-independent signaling involved. The mean level of aggregation response to ristocetin-vWF induced aggregation responses were in agitated CA6h versus WCA6hr also decreased from 100% fresh to $79.2 \pm 4.4\%$ versus $66.65 \pm 28.55\%$ after 48 hours storage ($p < 0.186$). Recall the ristocetin-vWF activation is mainly GPIb-dependent response, in vitro.

Spontaneous agglutinations were visually investigated. Activation properties were investigated by follow ups of aggregation curves integrities before and after agonists additions. No extraordinary differences were observed between samples to report.

Discussions

Platelets (PLTs) transfusion and their efficacy, CCI in a recipient depend on the storage conditions pretransfusion. In this study we investigate the effects of 6 hours interruption of agitation (metabolic resting) at 22–24°C, after 48 hours continue agitation versus metabolically active control PCs stored under continue agitation, in the permeable bags, in the Blood bank of Tehran.

We report here that compared to control group (CA6h), the PCs stored under agitation for 42 hours under standard condition at 22–24°C in permeable bags, and then rested for 6 hours (WCA6h) showed no significant differences in agonist induced-aggregation function. Both GPIb-dependent and independent signaling responses to 3 different activators which activate PLTs receptors via different signaling pathways responded almost the same. It was surprising for us that only ristocetin-vWF-GPIb interactions under a stir of 1000 rpm formed firm aggregates, and other 2 agonists (ADP, collagen), and their relevant receptors showed dramatic decrease to form firm aggregates. The differences between various samples were enormous, which might be caused by metabolic-dependency of responsiveness, metabolic dependency of signals transductions, donors' genotype and phenotype variability, however [4,10,22,23].

In other hand, it is assumed that continuous, gentle agitation of the PC maintains the pH levels throughout the period that the PLTs are stored at room temperature (22–24°C) in special, gas-permeable plastic bags to ensure their in-vitro quality and in-vivo effectiveness [24–26]. Moreover, to get the best results agitation should be either circular or flatbed movement because it affects the PCs quality [26]. It was, therefore, supposed that no agitation could negatively affect the quality and quantity of PCs prior to transfusion. However, others reported that a period of resting without agitation had positive effects on PLTs function and viability [13,25,26].

Here we report that compared to controls, interruption of agitation not only did not impair PLTs function but that it actually

had no negative effects on their responsiveness. Control group which continuously was under agitation showed no superior responsiveness to activators in old PCs.

Our hypothesis was based on the approach of Timori NH *et al* in 2013 introduced a novel technique to optimize the integrity of old PC during transportation. Timori NH *et al* used 6 hours interruption of agitation (metabolic resting) to preserve PLTs signaling function from stored PCs after 3 days. We did not expect such results. At first, we wonder why all (non-) agitated 48 hours stored PCs showed so dramatically decreased in aggregation responses? At second, why either CA6h or WCA6h showed the same (ir-) responsiveness and/or (de-) sensitivity to 3 different agonists? At least but not last how? And why GPIb-dependent signaling pathways responded adequately, while GPIb-independent pathways (collagen and ADP-receptors) did respond inadequately? The time and storage conditions were the same, except 6 hours interruptions of agitation, their metabolism, and oxidative phosphorylation were different. These aspects indeed need more investigations.

Based on different laboratories results, the collagen response and its (ir-) responsiveness and (de-)sensitization depend on calcium-dependent processes, [27,28]. which all are involved in primary haemostasis. Prolonged storage affects these processes and results in either mitochondrial damage or membrane lesions [11,29]. ADP-induced aggregation generally is weak response either in fresh or old PCs and their (ir-) responsiveness is depends on ADP receptors signaling [5]. Surprisingly, after 48 hours with or without agitation and/or active metabolic activity of PLTs, the ristocetin-induced aggregation and relevant signaling to PLTs (de-) sensitivity showed independency of continue, and/or interruption of agitation. How different PLTs receptors signaling work against agonist-induced aggregation after 48 hours continue agitation with or without 6-24 hours agitating conditions, needs more investigation.

Conclusions

We observed no significant deleterious effect on the GPIb-related signaling to respond to ristocetin-vWF to form firm aggregation, after interruption of agitation for 6 hours. Taken together, a short period of 6 hours non-agitating condition in the permeable bags had the same effect on PCs signaling and (ir-) responsiveness as continue agitation.

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agonists	CA6h	WCA6h	P-value	Normal range	CI
COLLAGEN	3.49 ± 1.73	3.46 ± 1.00	0.962	65.9%	1.30-1.36
ADP	4.30 ± 2.7	3.20 ± 3.9	0.518	76.4%	1.42-4.68
RISTOCITINE	79.2 ± 4.4	66.65 ± 28.55	0.186	74.5%	6.63-31.75

Table 1: In vitro agitated (n=10) and 6 hours non agitated (n=10) PLT variables.

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