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Prospective evaluation of platelets prepared by single and random methods during 5 days of storage: aspects related to quality and quantity

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Abstract

Platelets, which have an important role in hemostatic mechanisms and which were prepared by single and random methods were investigated to measure quantity, and aggregation response during 5 days of storage. The aggregation response and quantitative values of platelet concentrates (PCs), 60 of which were prepared by a single donor method and 62 by a random method were investigated during the 1st, 3rd, and 5th days of storage. The single donor platelets (SDP) were obtained by using the MCS Plus apheresis device and the random donor platelets (RDP) were obtained by two-phase centrifugation in the Heraeus 8500i centrifuge device (during the first phase, platelet rich plasma was obtained then platelet concentrate was obtained from this product) and were stored at 22 °C on a circular agitator. In addition, pH, PO_2, PCO_2, glucose and lactate values were measured in order to evaluate the effects of storage. The aggregation response was measured using adenosine diphosphate (ADP), epinephrine (EPN), collagen (COLL) and ristocetin (RIST). The cell count in mm^3 and the total cell count were also measured. The total cell counts and cells in mm^3 of the PCs which were prepared by the single donar method on the 1st, 3rd and 5th days, were: 3.11×10^{11}, 3.09×10^{11}, 3.07×10^{11} and 292×10^3, 290×10^3, 289×10^3 and of those prepared by the random method were: 5.71×10^{10}, 5.69×10^{10}, 5.66×10^{10} and 156×10^3, 153×10^3, 151×10^3. The mean aggregation responses of the PCs prepared by the two methods on the 1st, 3rd and 5th days, expressed as a % were: ADP: 94.8–93.2, 81.6–78.7, 44.3–8.2; COLL: 91.7–89.6, 79.2–74.2, 29.8–11.1; EPN: 88.5–91.3, 64.2–62.7, 39.4–4.5 and RIST: 89.4–89.4, 76.5–73.6, 14.4–3.2. Other data related to platelet storage were obtained by measuring the pH, PO_2, PCO_2, glucose and lactate levels of the PCs. In our study, it was determined that in spite of the optimal storage conditions, the aggregation response of the PCs decreased significantly, whereas, the numerical values changed little during the storage period.

Keywords: Platelets; Aggregation; Platelet counts; Single method; Random method

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1. Introduction

PCs play a primarily role in hemostasis [1]. Survival of platelets in the normal circulation is 9.5 days. When a PC transfusion is needed, it is possible to obtain the expected increments by considering two variables: (1) the blood volume of the transfused patient, and (2) the quality and the number of the transfused PCs [2]. In order to obtain sufficient numbers of functional platelets, both random donor platelets (RDP) and single donor platelets (SDP) are used. To supply SDP, only one donor is used, therefore transfusion complications – such as transfusion reactions, transmission of viral infections, and bacterial contamination – occur less frequently than with RDPs. Also, the alloimmunization risk decreases with SDP because the number of antigens to which the patient is exposed is less. In the SDP method, the number of the platelets that can be obtained from one donor is 6–8 fold greater than that of the other method; and the same donor, when necessary, can also be used again after 48–72 h. Also, because SDPs are often used without storage, then can be more effective.

But, SDPs require that the donor stay connected to the machine for a longer period, they are more expensive and require specialized equipment and personnel [3,4].

In this study, we investigated the aggregation response and the yield of platelets under appropriate conditions during 5 days of storage of platelets prepared by single and random donor methods using polyvinyl chloride + tri-2-ethylhexyl-trimellitate (PVC + TOTM) bags and which could be used for 5 days.

2. Materials and methods

From 09.01.2000 to 11.15.2000, in the Blood Bank of the Florence Nightingale Hospital, our study was performed with patients who had not taken acetylsalicylic acid in the past last 15 days, whose platelet count in was $\geqslant$150.000 mm^3 and whose platelet aggregation response to adenosine diphosphate (ADP), collagen (COLL) and ristocetin (RIST) were $\geqslant$70% and to epinephrine (EPN) $\geqslant$ 60%, on the 1st day of preparation of the platelet concentrate (PC). PCs, which were obtained by a SDP method from 60 people and by a RDP method from another 62 people, were used in the study. For the SDP method, 60 plateletpheresis units from 60 people (1 PU = 8 U of PCs) weighing between 225–330 g, were obtained; and for the RDP method, 62 PCs, weighing between 62–87 g were obtained from 62 people. The RDP method has two phases: in the first phase, the platelet-rich plasma (PRP) is isolated from the blood then, the PC is obtained by centrifugation.

In the SDP method, the PCs were obtained using the REF 994 E plust set of the Haemonetics MCS plus device which works on the principle of intermittent flow centrifugation and bags of CLX (Cuffer Co., USA), composed of PVC + TOTM.

In the RDP method, PCs were obtained by two-phase centrifugation of blood in PVC + TOTM blood bags (Kansuk Company, Turkey) using the Cryofuge 8500 I machine of the Heraeus Firm (Heraeus, Germany). In the SDP method, 50 cc of PC were transferred from the primary platelet bags, into the satellite bags which have a similar structure as the primary bag, with the assistance of the Steril Connection Device (SCD) (Terumo, Japan) 312. These bags, containing RDPs which were in their primary bags and the transferred SDPs, were stored on a circular agitator (Helmer, PAS 40 Model, Noblesvilla, USA) with 2–6 rpm as recommended in the literature [5].

The PCs were tested for aggregation response and numerical values on the 1st (the preparation day), 3rd, and 5th days. For calculations of total cell content the total gram weight, which the machine indicated automatically in plateletpheresis and was obtained by weighing with a sensitive balance in the random method. The cell count, was obtained using an automatic cell counter (Coulter, MD II model, USA), after diluting by 1/5. The resulting platelet counts were multiplied by 5, so that the platelet count in mm^3 was determined.

On each of the 3 days, the aggregation responses were studied using the Packs-4 (Helena Co., USA) machine as recommended by the manufacturer. The concentrations of the stimulants used were as follows: ADP: 20 μmol/ml, COLL: 5 μg/ml, EPN: 60 μmol/ml, and RIST:

500 μg/ml. 1800 μl of platelet and 500 μl of plasma from the products obtained during the PC collection, were used for each sample.

To evaluate the aggregation response, values above $\geqslant 70\%$ for ADP, COLL, RIST and $\geqslant 60\%$ for EPN were considered as a baseline. In order to check our results on aggregation and platelet counts, the samples from the first 5 PCs were also sent to the Cerrahpaşa Medical Faculty, Haematology Laboratory (for aggregation activities) and to Florence Nightingale Hospital, Biochemistry Lab. (for platelet counts) for assay at the same time. All of the results were similar and the study was continued. At the same time, in order to evaluate the storage conditions – throughout the follow-up period, the pH, PCO_2, glucose and lactate values were measured – in the Radiometer ABL 700 device (Radiometer Copenhagen Co., Denmark) at 37 °C and converted to values at 22 °C. The PO_2 value was converted to 22 °C in the Radiometer ABL 510 device (Radiometer Copenhagen Co., Denmark) [6].

Comparisons and statistical calculations between groups were made with the paired Student's *t*-test. Probability (P) values < 0.05 were considered significant. Results are expressed as mean ± standard deviation.

3. Results

The in vitro data from the PCs prepared by SDP and RDP methods related to their storage conditions over the 1–5 days are seen in Table 1.

When the quantitative and aggregation response parameters of the PCs prepared by these methods are examined, although the quantitative values in both of the methods decreased slightly showing no significance ($P > 0.05$) between the 1st and 5th days, by the 5th-day the values were above those accepted as a standard therapeutic dose, i.e., 3.04×10^{11} in SDP and 5.58×10^{10} in RDP. The aggregation values of the PCs decreased significantly ($P < 0.001$) from 1 to 5 days for both methods (Table 2).

When the two platelet preparations are compared quantitatively and for aggregation response, significant differences were determined in the numerical values as expected ($P < 0.001$), but no difference ($P > 0.05$) was found between the aggregation values (Table 3).

4. Discussion

In order to transfuse the best platelet preparations the patients in regard to both the total cell count and the quality; or to provide the optimal conditions for storage prior to transfusion, studies on both storage bags and platelet collection methods, have been carved out for years [7,8]. Instead of first generation bags made of PVC which allow platelets to be stored for 3 days, the second generation bags in which TOTM is added is the structure, were used. These PVC + TOTM bags, have an increased surface area and improved gas exchange and supply high levels of oxygen with less CO_2, available to alter consume the

Table 1
The in vitro data of PCs related to the storage conditions – which were prepared by single and random methods

Days	Method	Parameters				
		pH (Mean values at 22°)	PCO_2 (mm/Hg)	PO_2 (mm/Hg)	Glucose (mg/dl)	Lactate (mmol/l)
1	Single	7.35 ± 0.04	32 ± 6.35	22 ± 4.21	360 ± 14.5	0.9 ± 0.04
	Random	7.23 ± 0.04	31.7 ± 15.25	20.6 ± 3.88	405 ± 4.7	1.2 ± 0.02
3	Single	7.23 ± 0.08	12.1 ± 0.14	73.3 ± 10.7	356 ± 2.6	1.5 ± 0.13
	Random	7.18 ± 0.08	17.4 ± 2.36	36.2 ± 11.6	399 ± 14.7	3.11 ± 0.09
5	Single	7.08 ± 0.13	10.7 ± 1.59	83.6 ± 4.66	341 ± 6.42	3.7 ± 0.10
	Random	7.06 ± 0.13	13.9 ± 3.99	48.6 ± 9.67	384 ± 31.13	5.9 ± 03.99

Table 2
The dissociation of aggregation activation and the quantitative values of the PCs – prepared by single and random methods – on the 1st, 3rd and 5th days

Method	Single			Random		
Days	1	3	5	1	3	5
Mean values of						
Total cell (10^{11})[a]	3.11 ± 0.7	3.09 ± 0.7	3.04 ± 0.8	5.71 ± 1.8	69 ± 1.1	5.58 ± 0.6
Cell/mm^3 (10^3)	292 ± 71.3	290 ± 68.3	289 ± 35.5	156 ± 57.4	153 ± 52.7	151 ± 27.6
Statistical values	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Mean values of (as %)						
ADP (20 μmol/ml)	94.8 ± 11.5	81.6 ± 12.5	44.3 ± 7.2	93.2 ± 10.5	78.7 ± 9.6	8.2 ± 4
COLL (5 μg/ml)	91.7 ± 5.2	79.2 ± 21.3	29.8 ± 5.5	89.6 ± 8.4	74.2 ± 13.3	11.1 ± 3.7
EPN (60 μmol/ml)	88.5 ± 9.1	64.2 ± 14.3	39.4 ± 13.9	91.3 ± 3.8	62.7 ± 6.9	4.5 ± 6.5
RIST (500 μg/ml)	89.2 ± 6.8	76.5 ± 8.3	14.4 ± 4	89.4 ± 5.6	73.6 ± 10.7	3.2 ± 2.1
Statistical values	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

[a] (10^{10}) for random method.

Table 3
The comparison of the platelets which were prepared by single and random methods in respect of the quantitative and aggregation properties

Properties	Days	Method		*P* value
		Random Mean ± SD	Single Mean ± SD	
[a]TCC	1	$5.71 \times 10^{10} \pm 1.8$	$3.11 \times 10^{11} \pm 0.7$	$P < 0.001$
TCC	3	$5.69 \times 10^{10} \pm 1.1$	$3.09 \times 10^{11} \pm 0.7$	$P < 0.001$
TCC	5	$5.58 \times 10^{10} \pm 0.6$	$3.08 \times 10^{11} \pm 0.8$	$P < 0.001$
[b]C/mm^3	1	$156 \times 10^3 \pm 57.4$	$292 \times 10^3 \pm 71.3$	$P < 0.001$
C/mm^3	3	$153 \times 10^3 \pm 52.7$	$290 \times 10^3 \pm 68.3$	$P < 0.001$
C/mm^3	5	$151 \times 10^3 \pm 27.6$	$289 \times 10^3 \pm 35.5$	$P < 0.001$
ADP	1	93.2 ± 10.5	94.8 ± 11.5	$P > 0.05$
ADP	3	78.7 ± 9.6	81.6 ± 12.5	$P > 0.05$
ADP	5	8.2 ± 4	44.3 ± 7.2	$P < 0.001$
EPN	1	91.3 ± 3.8	88.5 ± 9.1	$P > 0.05$
EPN	3	62.7 ± 6.9	64.2 ± 14.3	$P > 0.05$
EPN	5	4.5 ± 6.5	39.4 ± 13.9	$P < 0.001$
COLL	1	89.6 ± 8.4	91.7 ± 5.2	$P > 0.05$
COLL	3	74.2 ± 13.3	79.2 ± 21.3	$P > 0.05$
COLL	5	11.1 ± 3.7	29.8 ± 5.5	$P < 0.05$
RIST	1	89.4 ± 5.6	89.2 ± 6.9	$P > 0.05$
RIST	3	73.6 ± 10.7	76.5 ± 8.3	$P > 0.05$
RIST	5	3.2 ± 2.1	14.4 ± 4	$P < 0.001$

SD: standard deviation.
[a] TCC: total cell count.
[b] C/mm^3: cell/mm^3.

buffering capacity of plasma, keep the pH stable; and cause a reduction in the glucose consumption and lactate production [8–11]. Despite these storage bags which extend the storage of platelets from 3 days up to 5 days and provided their activation remains optimal, factors such as: the manner in which the platelets are agitated during storage affect platelet membrane expression and the number

of polymorphonuclear leukocytes in the platelet concentrates are some of the reasons for the problems seen during storage of the platelets [12]. It was reported that aggregation of the platelets to agents like ADP, RIST and EPN decreased depending on the storage period [13–16]. The, basic principle in preparing RDPs is sedimentation, therefore two-phase centrifugation may affect many activation markers on the surface membrane, the morphology, and the function of the platelets; on the other hand, apheresis platelets are subjected to intermittent or continuous flow centrifugation during collection but are not sedimented. The type of anticoagulant (acid citrate dextrose [ACD] in one system and citrate phosphate dextrose adenine [CPDA] in the other) added to the PC and the way in which it is mixed with blood also differ for these two types of PCs; therefore, these differences during preparation of the RDP and SDP may cause differences in the quality of the platelets [17].

In our study, the in vitro data, related to the storage process of the PCs which were prepared by two different methods, indicated that they had optimal conditions. In spite of this, when the two methods were compared in regard to aggregation activity, it was determined that there were no differences between them on the 1st and the 3rd days.

Sloand et al. [7] obtained similar conclusions in an investigation where the two methods were compared; it was reported that there were no differences between the two methods in respect to the aggregation by ADP and COLL on the lst and 3rd days but a difference was determined on the 5th day. These results indicate that, the PCs, which were prepared by the two methods, have similar rates of platelet aggregation compared with each other until the 3rd storage day; but on the 5th day, the activity was found to be much lower than normal for both of the methods with the values of the SDP method higher than that of the RDPs. When we examine aggregation response throughout the storage period: the response to ADP, EPN, COLL and RIST decreased significantly on the 3rd day in both of the methods, but still remained above the normal values; however, it decreased on the 5th day compared to both the 1st and 3rd days.

Investigations in different years, by Koerner [18] in Germany, Turner et al. [19], and Rock et al. [15,16] from Canada, reported that the aggregation response to ADP and COLL, ADP and COLL/EPN, ADP and EPN, respectively, decreased gradually from the 1st day to the 5th day.

The results of our in vitro study were parallel to the conceptions in the literature. On the other hand, according to the in vivo studies of three investigators, although the platelet activation values decreases in vitro until the 5th day, they regained activity in the circulation in the ratios of 30%, 37–40%, 67%, 65% in order for four investigations in which the platelets were transfused to volunteer thrombocytopenic patients after the 5th storage day. Therefore, they concluded that the in vivo recovery of platelets occurs although storage lesions have developed related to the in vitro conditions and the platelets then regained their activation capacities [13–16]. But as stated in two other investigations [20,21], how effective would the platelets be for treating acute hemorrhage perioperatively, when used after having lost the property of aggregation because of storage. This was a subject of considerable discussion.

As a result, in spite of the optimal storage conditions, aggregation responses of the platelets which were prepared by two methods, decreased significantly in the course of time throughout the storage process; however there was an insignificant decrease in numerical values.

From these results, we consider that, in order to provide more clinical benefit for the patients, the platelets should be transfused as soon as possible during the storage period. We also suggest that further studies, including in vivo investigations, are also necessary in order to reveal the clinical benefit provided by the stored platelets on the patients.

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