Effect of Cold Exposure on Platelet Concentrates: Changes in Platelet Indices and Aggregation States

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Abstract

Samples from platelet concentrates (filtered/non-filtrated, at the begining/the end of shelf life) were exposed to 4°C overnight, subsequent to dilution in platelet storage media (PSM) and/or exposure to EDTA to induce shape changes. Paired sampling protocol (± EDTA) was used and changes in cellular indices and induced-aggregation states were measured by Technicon H*1. Cold induced changes in platelets as identified by increase in MPV (0.5-0.8 fL for EDTA; 1.5-2.0 fL for citrated samples) with concomitant reverse changes in PDW ranging from 2-13 per cent was observed. Processing, storage and cold exposure also induced disparity between leucocyte peroxidase/basophil counts. This in conjunction with changes in platelet counts and cellular indices upon exposure to EDTA provide a unique new tool for assessing the aggregation states of platelets during processing and storage. Both filtration and dilution in PSM affect platelet storage stability. Platelets which have already undergone shape changes (i.e. exposure to EDTA) responded to a lesser degree to cold exposure. Our findings indicate that platelets' response to cold exposure can be used as a simple, reliable and accurate test for assessment of platelet morphological and function integrities.

Platelets are known to be extremely sensitive to changes in the temperature of their microenvironment. Prolonged exposure to cold (4°C) reportedly leads to irreversible loss of platelet discoid shape, attributable to depolymerisation of microtubuli and breakdown of platelet contractile protein[1]. Recently we reported that such morphological changes can be easily monitored by automated cell counters[2] in particular those based on light scattering principles such as Technicon H*1. In this study, we have evaluated the effect of cold exposure (4°C, overnight) on platelet indices and aggregation states, using paired sampling protocol[3]. The potential contribution of leucocyte-in-

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duced platelet morphological changes during storage as well as activation of platelets induced by their resuspension in platelet storage media and/or EDTA were also investigated by using filtrated and non-filtered platelet concentrates (PC) of the same origin at the begining (day 2) and at the end of shelf life (day 5).

MATERIAL AND METHOD

Platelet concentrates were prepared by pooled buffy coat (4 units) technique using Optipress system (Baxter) according to described validated protocol[4] and stored in 1 litre PL-732 platelet storage bag (Fenwal).

Six packs of PC were used. Two packs of PC with the same ABO group were pooled together and equally subdivided. One was filtered using Sepacell PL-5 (Asahi), the post-filtered was collected in a new PL-732 bag, and then kept in identical conditions to the non-filtered product in Helmer Shaker at 22°C.

Samples were taken from each product at the begining (day 2) and at the end of shelf life (day 5). In each case, they were diluted with PSM (Baxter) at 1/2, 1/3 and 1/4, dilutions. Both neat and dilutes samples were exposed to EDTA at concentration of 30 mM according to the established paired sampling protocol (± EDTA)[3]. Then after 1 hour storage at room temperature. Each of the citrated and EDTA samples were subdivided into two portions. One was kept at room temperature (22°C), the other was kept at 4°C. Both sets of samples were kept overnight.

Technicon H*1 was used for the assessment of platelet count (PLT), size distribution indices, morphological changes and leucocyte count (by peroxidase channel - WBCp and basophil channel - WBCb).

For spontaneous aggregation three millilitres of citrated PC (fresh/stored) were put in a plastic tube which was then put on a rotating mixer and sampled sequentially every 2 minutes for cell counting.

Data are expressed as mean of three pairs of experiments.

RESULTS

Characteristic properties of filtered/non-filtered PC upon storage

The characteristic properties of day 2 filtered PC, kept at room temperature were similar to those of non-filtered PC with slight changes in mean platelet volume/platelet distribution width (MPV/PDW). However, on day 5 a much sharper fall in MPV with concomitant increase in PDW was noted as shown in Table 1. Cold exposure had a dramatic effect on MPV and PDW of both filtered and non-filtered PC. Although at room temperature the storage-induced changes in MPV/PDW of filtered products were different from non-filtered products (day 5), their response to cold was practically identical, suggesting platelets have maintained their overall structural integrity.

Effect of cold exposure on EDTA samples

At room temperature, storage-induced changes for filtered products appeared to be greater than non-filtered products which showed no change (MPV day 2 *vs* day 5: 6.4fL *vs* 6.1fL and 6.5fL *vs* 6.5fL for filtered and non-filtered products respec-

Table 1. Effect of cold exposure on citrated samples (n = 3)

	Day 2				Day 5			
	MPV (fL)		PDW (%)		MPV (fL)		PDW (%)	
	Filtered	Non-Filtered	Filtered	Non-Filtered	Filtered	Non-Filtered	Filtered	Non-Filtered
RT*	6.6	6.7	60.2	59.4	6.3	6.7	65.0	60.7
4°C	8.4	8.5	47.8	47.4	8.3	8.7	51.9	47.7
**Diff.	(1.8)	(1.8)	(-12.4)	(-12.0)	(2.0)	(2.0)	(-13.1)	(-13.0)

* Room temperature (22°C)
** (4°C) - (RT) = Diff

Table 2. Effect of cold exposure on EDTA-exposed samples (n = 3)

	Day 2				Day 5			
	MPV (fL)		PDW (%)		MPV (fL)		PDW (%)	
	Filtered	Non-Filtered	Filtered	Non-Filtered	Filtered	Non-Filtered	Filtered	Non-Filtered
RT*	6.4	6.5	55.4	55.4	6.1	6.5	56.3	55.5
4°C	7.2	7.3	51.1	50.1	6.7	7.0	53.9	53.5
**Diff.	(0.8)	(0.8)	(-4.3)	(-5.3)	(0.6)	(0.5)	(-2.4)	(-2.0)

* Room temperature (22°C)
** (4°C) - (RT) = Diff

Table 3. Effect of cold exposure on sample diluted in PSM (n = 3)

Citrated Samples	MPV (fL)											
	Day 2						Day 5					
	Filtered			Non-Filtered			Filtered			Non-Filtered		
Dil.	RT*	4°C	** Diff.	RT*	4°C	** Diff.	RT*	4°C	** Diff.	RT*	4°C	** Diff.
Neat	6.6	8.4	(1.8)	6.8	8.5	(1.7)	6.3	8.3	(2.0)	6.7	8.7	(2.0)
1/2	6.6	8.1	(1.5)	6.6	8.1	(1.5)	6.2	8.1	(1.9)	6.6	8.3	(1.7)
1/3	6.5	8.2	(1.7)	6.6	8.2	(1.6)	6.6	8.2	(1.6)	6.9	8.5	(1.6)
1/4	6.4	8.1	(1.7)	6.6	7.9	(1.3)	6.5	8.2	(1.7)	6.9	8.3	(1.4)
EDTA Samples	MPV (fL)											
	Day 2						Day 5					
	Filtered			Non-Filtered			Filtered			Non-Filtered		
Dil.	RT*	4°C	** Diff.	RT*	4°C	** Diff.	RT*	4°C	** Diff.	RT*	4°C	** Diff.
Neat	6.4	7.2	(0.8)	6.5	7.3	(0.8)	6.1	6.7	(0.5)	6.5	7.0	(0.5)
1/2	6.6	7.2	(0.6)	6.7	7.3	(0.6)	6.5	7.0	(0.5)	6.7	7.1	(0.4)
1/3	6.8	7.3	(0.5)	6.8	7.3	(0.5)	6.7	7.1	(0.4)	7.0	7.3	(0.3)
1/4	6.9	7.4	(0.5)	7.4	7.4	(0)	6.3	7.0	(0.7)	7.0	7.4	(0.4)

* Room temperature (22°C)
** (4°C) - (RT) = Diff

tively). The response to cold exposure of both products were equivalent but differed between day 2 and day 5. This is indicative of the initial storage-induced discoid/spheric conversion of citrated samples attributed to the platelet response to EDTA and cold exposure. A similar but reverse pattern of changes was observed in PDW as shown in Table 2.

Effect of cold exposure on samples diluted in PSM

Comparative analysis were made between samples from day 2 and day 5 (filtered/non-filtered) PC at 3 dilutions (1/2, 1/3 and 1/4). The results are summarised in Table 3. The patterns of changes in MPV of citrated samples upon dilution were the same for filtered and non-filtered products, but dif-

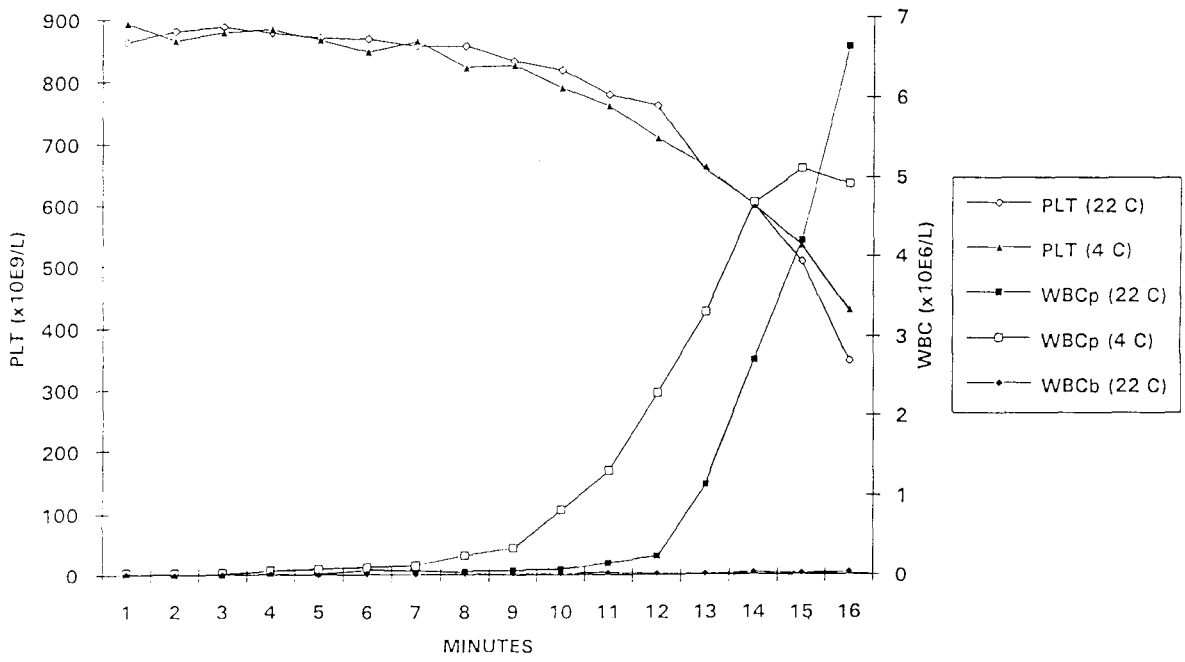

Fig. 1. Spontaneous aggregation of day 1 platelet concentrate, stored either at room temperature (22°C) or 4°C, showing the fall in platelet counts (◇, ▲) is associated with increase in WBCp (■, ❐) with no change in WBCb (◆). Note the shortening of the lag phase in 4°C stored sample as compared to 22°C stored sample of the same origin.

fered for day 2 and day 5 products. In the former, MPV were slightly decreased, whereas, in the latter they were increased in the highest dilution (Table 3). However, both day 2 and day 5 products had similar response to cold exposure though the response was slightly higher for the neat preparation.

In the EDTA samples MPV gradually increased upon dilution on day 2 with no clear cut pattern on day 5. Whereas, in all samples there was a systematic decrease in their response to cold exposure upon dilutions as indicated by differences in the MPV between room temperature and 4°C.

Effect of cold exposure on spontaneous aggregation and disparity between WBCp/WBCb

Fresh PC (day 1) do undergo spontaneous aggregation upon sequential counting. This is shown by the fall in platelet count and concomitant increase in WBCp while no change in WBCb occurred. The representative pattern of cold-induced spontaneous aggregation as compared to 22°C is shown in Fig. 1 indicating that cold exposure accelerated the rate of spontaneous aggregation.

The lag phase of spontaneous aggregation is gradually prolonged upon storage when kept at 22°C. However, the cold preparation maintained this pattern up to the end of shelf life (not shown). These findings suggest that the disparity between WBCp/WBCb is a good index of the states of platelet aggregation.

The effect of pre-storage leucocyte reduction on changes in activity states of platelets was also investigated at both room temperature and 4°C. Table 4 summarises the results of WBCp for day 2 and day 5 products. On day 2 there was a slight increase in WBCp subsequent to cold exposure,

Table 4. Effect of cold exposure on disparity between WBCp/WBCb (n = 3)

	WBCp/WBCb (x10^6/L)			
	Day 2		Day 5	
	Filtered	Non-Filtered	Filtered	Non-Filtered
RT*	0.03/0.02	0.34/0.34	0.07/0.02	0.29/0.30
4°C	0.07/0.01	0.42/0.31	1.84/0.01	1.17/0.30

* Room temperature (22°C)

whereas, on day 5 there was a substantial increase in WBCp. The increase in WBCp of filtered products was much higher than non-filtered products of the same origin.

DISCUSSION

Changes in platelet morphology, structure and function, during storage of platelet concentrates have been well documented[2,5,6]. These changes may reflect the ageing processes, as well as, processing induced cellular injury. Both the presence of leucocytes in platelet concentrates during storage and prolonged exposure to cold (4°C) appear to effect platelet morphological and functional integrities as evaluated by both *in vitro* tests and platelet survival *in vivo*[4,7-9]. Therefore, leucocyte filtration has become popular in a transfusion setting and every effort has been made to maintain whole blood prior to processing for platelets at 22°C.

Unfortunately, until recently, there was no simple and appropriate procedure which could reflect platelet functional and morphological integrities during cold exposure. Recently, we reported that the use of paired sampling (± EDTA), followed by cell counting provides a useful index of the platelet aggregation states and platelet functional integrity comparing well with other *in vitro* tests[3]. The application of a similar principle to cold exposure has been evaluated in this manuscript.

The results of this study showed that cold shock can be used as a simple and cost effective test to assess the activity states of platelets. The degree of cold-induced shape changes and platelet aggregation states appear to be related to initial morphological and activation states of platelets. In this respect filtration appears to induce some storage injury. This is experimentally demonstrated in Fig. 1 indicating that cold exposure clearly leads to shortening of lag phase of spontaneous aggregation. Furthermore, samples which have already undergone activation and pseudopodia formation due to prolonged EDTA exposure do respond to a lesser degree to cold exposure than their counterparts.

It should be noted that rapid onset of cold-induced platelet shape changes occurs even within two hours, however, these changes appear to be reversible unless the samples have undergone activation and fragmentation.

The replacement of plasma protein with PSM has been introduced as a measure for reducing the transfusion reactions and improving platelet longevity. However, according to some authors[4], a certain ratio of plasma to PSM can facilitate the thrombin generation contributing to platelet shape changes and clumping. Although the design of our experimental protocol differs from the condition employed by Hogman (i.e. in our protocol the platelet count will drop upon dilutions) nevertheless the observed changes in platelet indices in PSM are indicative that the presence of plasma components may be essential to keep the platelet in its native shape and PSM may contribute to some platelet injury and/or clumping.

The practical implication of our findings to operational aspects and quality assurance programme of platelets is enormous. Firstly, care is needed during transport of blood for processing, as even short exposure to cold will lead to platelet clumping, affecting both platelet recovery from such a unit as well as leading to erroneous results, in respect to platelet size and leucocyte content, in particular when WBCp is recorded as the measure of leucocyte content. Secondly, it helps in identification of some short comings in platelet collection,

processing and/or storage-induced platelet injury as platelets which have already undergone shape changes loose their ability to respond to cold. This is exemplified by activation and pseudopodia formation, followed by swelling and fragmentation induced by prolonged (overnight) platelet concentrate in EDTA.

The potential beneficial advantage of overnight temperature recycling from 4°C to 37°C has been reported[10]. This is substantiated by the facts that fresh PC and those which have not completely undergone fragmentation (i.e. maintain their glycolytic integrity *p*H > 6.4) can return almost completely to their original shape as indicated by their size distribution pattern upon warming (not shown). Recently, evidence was presented that rewarmed PC (22°C *versus* 37°C) have a better *in vivo* property.

Currently the short survival of PC produced and stored at 4°C precludes their current clinical usage. Hence, technical/clinical challenges remain to reduce the cold storage injury.

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ผลของอุณหภูมิต่ำต่อการเปลี่ยนแปลงของเกร็ดโลหิตเข้มข้น

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เกร็ดโลหิตมีความไวต่อการเปลี่ยนแปลงของสิ่งแวดล้อม การศึกษาเกร็ดโลหิตจากเกร็ดโลหิตเข้มข้นซึ่งผ่านการกรองและไม่ผ่านการกรองเอาเม็ดโลหิตขาวออก ที่วันแรกและวันสุดท้ายของอายุการเก็บโดยเก็บไว้ที่อุณหภูมิห้องและที่ 4°C และเจือจางด้วยสารละลายที่ใช้เก็บเกร็ดโลหิตเข้มข้น โดยวิธีการดูความแตกต่างระหว่างตัวอย่างที่ผสม EDTA และที่ปราศจาก EDTA และโดยการใช้เครื่อง Technicon H*1 ในการศึกษาความเปลี่ยนแปลงของค่าพารามิเตอร์ต่างๆ ได้แก่ ค่าเฉลี่ยของปริมาตรของเกร็ดโลหิต ค่าความเบี่ยงเบนของปริมาตรของเกร็ดโลหิต ค่าผลต่างระหว่างจำนวนเม็ดโลหิตขาวเปอร์รอกซิเดส/เบโซฟิล และจำนวนเกร็ดโลหิต พบว่าภาวะอุณหภูมิที่ต่ำลงทำให้มีการเปลี่ยนแปลงของเกร็ดโลหิต โดยพบว่าค่าเฉลี่ยของปริมาตรของเกร็ดโลหิตเพิ่มขึ้น 0.5–0.8 fL และ 1.5–2.0 fL ในตัวอย่างที่ผสม EDTA และที่ปราศจาก EDTA ตามลำดับ ในขณะเดียวกันพบว่าค่าความเบี่ยงเบนของปริมาตรของเกร็ดโลหิตเพิ่มขึ้นร้อยละ 2–13 พบการเปลี่ยนแปลงในผลต่างระหว่างจำนวนเม็ดโลหิตขาวเปอร์รอกซิเดส/เบโซฟิล นอกจากนี้ยังพบการเปลี่ยนแปลงของจำนวนเกร็ดโลหิต ผลที่ได้จากการศึกษานี้แสดงถึงการตอบสนองของเกร็ดโลหิตต่ออุณหภูมิที่ 4°C สามารถนำมาใช้ศึกษาการเปลี่ยนแปลงของรูปร่างและหน้าที่ของเกร็ดโลหิต โดยเป็นวิธีที่มีความสะดวกถูกต้องและเชื่อถือได้

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