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Effects of Storage Duration and Volume on the Quality of Leukoreduced Apheresis-derived Platelets: Implications for Pediatric Transfusion Medicine

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Abstract

Background—Platelet storage adversely affects platelet structure and function *in vitro*, and is associated with decreased platelet recovery and function *in vivo*. In pediatric transfusion medicine, it is not uncommon for small residual volumes to remain in parent units following aliquot preparation of leukoreduced apheresis-derived platelets (LR-ADP). However, limited data exists regarding the impact of storage on residual small volume LR-ADP.

Study Design and Methods—Standard metabolic testing was performed on residual volumes of LR-ADP following aliquot removal and platelet aggregometry using a dual agonist of ADP and collagen was performed on stored, small volume aliquots (10-80 ml) created from an *in vitro* model of platelet storage.

Results—77 LR-ADP underwent metabolic (n=67) or metabolic and aggregation (n=10) studies. All products maintained a pH > 6.89 throughout storage. Lactate and pCO₂ increased proportionally with longer storage time. Regardless of acceptable metabolism during storage, aggregation in 10-20 ml aliquots was impaired by day 4 and aliquots less than 40 ml demonstrated the most dramatic decrease in aggregation from baseline.

Conclusions—Despite maintenance of acceptable metabolic conditions, residual volumes of LR-ADP develop impaired aggregation *in vitro* that may adversely affect platelet survival and function *in vivo*. At volumes below 40 ml, LR-ADP revealed reduced aggregation. As a result, it is recommended to monitor and record volumes of LR-ADP used for pediatric transfusion. Moreover, once LR-ADP attain a volume of 50 ml or less on day 4 or 5 of storage, consider discarding these products until their *in vivo* efficacy can be studied.

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Keywords

residual apheresis platelet volumes storage; platelet metabolism and function; pediatric transfusion of platelets

Introduction

From donor collection to patient transfusion, platelets acquire deleterious alterations in shape, structure, and function *in vitro* encompassing the platelet storage lesion that may result in decreased hemostatic function as well as shortened survival *in vivo*¹. Consequently, improvements to storage conditions have been extensively investigated over the past decade, including, but not limited to, modifications in donor collection techniques, container composition, and storage media to maximize platelet viability and function.

Despite these advances, metabolic derangements continue to occur, most often involving extreme conditions of pH. Decreases in pH result from increased CO₂ production, as well as, lactate generation at the expense of glucose consumption. Increases in pH have also been shown to adversely impact platelet function *in vivo*². Additionally, platelets demonstrate signs of *in vitro* activation as evaluated by indirect measurement of alpha granule release by determination of P-selectin (CD62P) expression and reduced aggregation response to various *in vitro* agonists including ADP, collagen, epinephrine, and ristocetin³.

In pediatric transfusion medicine, small volume transfusion is routine practice and provision of aliquots from LR-ADP has become commonplace. Use of LR-ADP minimizes exposure often to only a single donor thereby limiting the risks of transfusion-transmitted disease and alloimmunization. Furthermore, aliquot component preparation from LR-ADP is an essential process for optimal utilization of inventories in hospital based pediatric transfusion services. Nonetheless, production of these aliquot products may compromise platelet viability and function, and studies focusing on the storage lesion associated with these residual, small volume components are limited. Quality monitoring is therefore critical for the assurance of a viable and functional platelet product. To this end, the present study was designed to investigate the metabolic parameters and platelet aggregation profiles of small residual volumes of LR-ADP after aliquot allocation for patient transfusion and small volume products created from an *in vitro* model of platelet storage.

Study Design and Methods

Platelet Preparation and Storage

LR-ADP were obtained from one of the following blood suppliers: the American Red Cross Blood Services, Southern Region (Douglasville, GA), LifeSouth Community Blood Center (Gainesville, FL), and Bonfils Blood Center (Denver, CO). No donor collection information was available for retrospective review. Two types of storage containers were used by suppliers and recorded: Baxter PL2410 (Fenwal, Inc., Lake Zurich, IL) or Gambro ELP (CaridianBCT, Lakewood, CO).

In phase I experiments that began as part of a quality control initiative, only LR-ADP that achieved a residual volume of 30-90 ml following aliquot removal for routine patient transfusion at days 4 or 5 post-collection were included. For *in vitro* studies, 1-2 ml was removed from each LR-ADP using a transfer pack to obtain a complete blood count (Coulter® LH 750 analyzer, Beckman Coulter, Fullerton, CA). Subsequently, a small volume of platelets flowed into the transfer pack tubing and was removed for biochemical analysis. In phase I experiments, these residual small volume LR-ADP were available for

patient transfusion; however, indications for transfusion and outcomes following transfusion with the exception of a post-transfusion platelet count obtained within 24 hours of transfusion were unknown.

During phase II experiments, an *in vitro* model of platelet storage was created. In this model, a single LR-ADP was divided into various volumes in identical storage containers and maintained at that volume from receipt until expiration of the LR-ADP. Upon receipt of the LR-ADP, day 2 or 3 post collection, baseline metabolic and platelet aggregation studies were performed. More specifically, for baseline testing, 1-2 ml was removed from each LR-ADP using a transfer pack to obtain a complete blood count and subsequent to this collection, a small sample was used for metabolic and aggregation studies. Immediately following removal of sample for baseline testing, aliquots of various volumes ranging from 10 ml to 80 ml were transferred into a polyolefin platelet storage container (PL2410, Baxter Healthcare Corporation, Deerfield, IL) using a sterile connecting device to closely mimic original storage conditions. After a desired volume was obtained, the bags were sealed and placed on an agitator (Helmer, Inc., Noblesville, IN) at $22 \pm -2^{\circ}\text{C}$. For respiration, all inner surface area was wet with the volume of the product in the LR-ADP aliquot. Of note, multiple aliquots of similar volumes were made from several LR-ADP. On each subsequent days leading up to the expiration of the product (maximum 5 days), the above procedure for ancillary testing was repeated and aliquots were discarded at expiration and, therefore, not transfused.

In Vitro Measurements

Biochemical Analyses—Blood gas measurements and pH determination were performed using a point of care instrument (iSTAT 1 analyzer, iSTAT Corporation, East Windsor, NJ). Metabolic parameters recorded included pH, pCO_2 , pO_2 , bicarbonate (HCO_3), and lactate.

Functional Analyses—For platelet aggregation studies, a portion of the sample was centrifuged at 2500 rcf for 15 minutes in order to obtain platelet poor plasma (PPP), of which 250 μL was used as a negative control for the platelet aggregometer (AggRAM, Helena Laboratories, Beaumont, TX). Commercial pooled normal plasma (Precision BioLogic, Dartmouth, Nova Scotia) was used to adjust the platelet count in the sample to a final concentration of $200 - 300 \times 10^3$ platelets/ μL . For testing, 225 μL of the diluted sample was pipetted into cuvettes containing a metallic stir bar, and incubated at 37°C for 1-3 minutes in the platelet aggregometer. Thereafter, 25 μL of a 1:1 mixture of 10 micromolar (μM) ADP and 10 micrograms per ml ($\mu\text{g/ml}$) collagen was added to the platelet rich plasma (PRP) for a total volume of 250 μL . Aggregation was measured by determining the optical density which is converted into percent activity over the ten minute period after the combination of agonists was added. Maximum aggregation was recorded and a response of 70% was determined to be adequate; this cut-off is used for clinical diagnostic testing at our institution as a guideline in the absence of a laboratory determined reference range and no standardized testing recommendations. In addition, the percent difference from baseline (day 2 or 3 dependent upon receipt of LR-ADP) to day 5 of storage was determined.

Statistical Analysis—Ranges, means and standard deviations (SDs) are reported. Paired t tests were performed, and a p-value < 0.05 was considered statistically significant. Data recording and descriptive statistical calculations used Excel (Excel 2007, Microsoft Corporation, Redmond, Washington).

Results

Effect of storage duration and volume on platelet metabolism of residual small volumes following aliquot preparation for patient transfusion

In phase I experiments, metabolic studies were performed on a total of 67 LR-ADP on day 4 (n=22) and day 5 (n=45) post collection (table 1). The residual volumes ranged from 30 to 90 ml in both groups with a mean volume of 61.6 ml and 61.9 ml on days 4 and 5, respectively. In 4 day old LR-ADP, platelet counts ranged from 8.83×10^5 to 1.53×10^6 with a mean platelet count of 1.21×10^6 while platelet counts of 5 day old units averaged 1.23×10^6 (6.23×10^5 - 1.58×10^6).

These small volume LR-ADP aliquots maintained a pH above 7.1 in all units with a mean pH of 7.48 (7.27-7.62) and 7.42 (7.13-7.65) on days 4 and 5 of storage, respectively. Additionally in these units, CO₂ and O₂ remained relatively constant. However, in 5 day old platelets, lactate concentrations were significantly increased, 104.8 mg/dl (72-157.5 mg/dl) as compared to 86.2 mg/dl (43.7-106.8 mg/dl) in 4 day old platelets (p<0.0001). In addition, there was no significant difference in pH, CO₂, O₂, and lactate concentrations between Baxter PL2410 and Gambro ELP storage containers. In these phase I experiments, 19 of 45 residual volume aliquots were transfused with a resultant mean increase in platelet count of 27,900/ μ l (range -77,000 – 141,000) determined from pre and post-transfusion platelet counts. Only two patients did not experience an increase in platelet count following transfusion; however, the clinical circumstances were unknown.

Effect of storage duration and volume on platelet function using an in vitro model of platelet storage

In phase II experiments, small volume components were created from a total of 10 LR-ADP. In all aliquot products, platelet counts remained relatively constant over 5 days of storage (table 2). The *in vitro* metabolic data is provided in Table 3 and results were comparable to those obtained from initial metabolic experiments using remaining volumes following removal of aliquots for patient transfusion. pH remained acceptable (>6.2) in all aliquots with the smallest volume aliquots (10-20 ml) having the lowest pH (range 6.78-7.32) on day 5. As expected, CO₂ and bicarbonate concentrations decreased in all platelet units, while lactate concentrations increased throughout the storage period, albeit to a greater extent in smaller volumes.

Results of *in vitro* platelet aggregation studies are shown in Table 4. At five days of storage, aliquots ranging in volume from 50 to 80 ml aliquots maintained a maximum aggregation of 70% using the paired ADP/collagen agonists. Contrastingly, the *in vitro* platelet aggregation of the smaller volume LR-ADP aliquot products of 40 ml and less decreased below 70% and had the greatest change in aggregation from baseline of 25.1% and 35.8% in 30-40 ml aliquots and 10-20 ml aliquots at five days, respectively. Of note, the LR-ADP from which 10-20 ml aliquots were made had a residual volume of 260 ml and in this parent unit there was no impairment in platelet aggregation from baseline.

Conclusion

The literature on platelet storage lesions has primarily investigated the metabolic changes and acquired platelet function defects that accrue in both LR-ADP and random donor platelets following storage and focused on the feasibility and safety of the extension of the expiration of platelets to seven days with concomitant mandated bacterial screening^{2,4}. However, once platelet units are manipulated *ex vivo*, which is regularly performed in pediatric transfusion medicine, changes can transpire which further influence platelet viability and function, and studies concentrating on the storage lesion associated with these

residual, small volume components are limited. As a result, this study was designed to investigate changes in metabolism and platelet aggregation of small residual volumes of LR-ADP after aliquot allocation for patient transfusion and small volume products created from an *in vitro* model of platelet storage. The results indicate reduced volume LR-ADP maintain acceptable metabolic profiles over 5 days of storage and maximum aggregation is maintained with as little as 50 ml present in the original storage bag. More specifically, platelet aggregation was impaired in aliquots with volumes of 10-40 ml with decreases in aggregation from baseline of 25.1% in 30-40 ml aliquots and 35.8% in 10-20 ml aliquots despite maintenance of platelet counts and acceptable metabolic conditions (pH, pCO₂, HCO₃, lactate concentrations).

In phase I experiments, metabolic studies were performed on residual volumes of LR-ADP following aliquot removal for patient transfusion to better characterize these changes as they occur in real time practice. Whereas, phase II experiments were performed using an *in vitro* model of platelet storage designed to control for the effect of storage on volume. In this *in vitro* model, baseline testing was not performed until day 2 or 3 post-collection when LR-ADP were received in the hospital blood bank. Consequently, it is difficult to fully appreciate the potential difference in platelet aggregation from collection to expiration in these small volume aliquots. Additional limitations include the inability to exclude collection method as a variable responsible for changes in platelet aggregation over duration of storage and not having access to a full volume LR-ADP from the same donor to control for variability in aggregation response.⁵ Therefore, only one experiment had an almost full LR-ADP as a control.

Few studies have specifically investigated the metabolic characteristics of small volume platelet aliquots^{2,6,7}. Dumont et al compared metabolic characteristics in four groups of LR-ADP including two with reduced volumes (mean volumes of 120 ml and 113 ml compared to 333 ml and 307 ml) over seven days of storage and found gradual decline in *in vitro* metabolic measurements (pH, pCO₂, pO₂, bicarbonate, glucose, lactate, and O₂ consumption) throughout storage; however, characteristics of all components were acceptable and there was no difference between volumes². Similarly, van de Meer et al studied the metabolic characteristics of LR-ADP split into 3 or 4 aliquots for pediatric transfusion prior to storage⁶. The authors concluded that these small volumes that averaged 69 ml can be acceptably stored for up to 5 days duration; however, no platelet function studies or post-transfusion outcomes were investigated. Lastly, Strasser et al investigated the metabolic characteristics of LR-ADP aliquots obtained in either a 30 ml bag, 60 ml bag, or 50 ml syringe for neonatal transfusion⁷. These split products were produced from LR-ADP at different time points over storage (days 1-4) and stored for 2 to 4 hours. In these split products, there was no impairment of pO₂, lactate concentration or pH despite decreased platelet concentrations.

However, unlike preservation of metabolic function, platelet aggregation has consistently been shown to decrease over storage in both random donor and LR-ADP products⁸⁻¹¹. While these reports have exclusively used single agonists for platelet function testing, it has been argued that using pairs of agonists during *in vitro* function testing may optimally recreate *in vivo* conditions as physiologically platelet aggregation occurs in response to the synergistic action of a combination of agonists at sites of injury^{12,13}. As a result, we deliberately decided to use a mixture of ADP and collagen as agonist.

Moreover, there is limited literature that evaluates platelet function of small volume LR-ADP aliquots. One publication has demonstrated that the temporary storage of leukoreduced platelet concentrates in gas impermeable syringes has had no adverse effect on platelet function or increased platelet activation for up to a six hour storage period. This outcome

resulted despite the volume reduction of some units along with decreases in pH with reciprocal increases in lactate concentration^{14,15}. Additional studies have confirmed these findings⁷. Furthermore, volume reduction of LR-ADP, a common practice in neonatal and pediatric transfusion medicine, has been reported to result in increased spontaneous *in vitro* platelet activation with resultant decreased aggregability¹⁶. Still, another publication has shown that platelet components with altered volumes maintained under original storage conditions have acceptable pH concentrations with the current FDA and AABB standards and have no statistically significant difference in the improvement of post-transfusion platelet increments when compared to unaltered volume products, suggesting that these manipulated products may be as efficacious¹⁷.

Despite impaired aggregation *in vitro*, transfusion of platelets with metabolic derangements accrued over 5 days of storage has resulted in recovery of platelet function *in vivo*^{18,19}. Similarly, decreases in pH below 6.2 were not associated with any deficit of *in vivo* platelet recovery or survival²⁰. Thus, *in vitro* diagnostic testing such as pH and platelet functional assays continue to serve as the only surrogate markers for *in vivo* platelet survival and function, each with their own limitations. Nevertheless, impaired aggregation at volumes below 40 ml may result in decreased *in vivo* function and viability, warranting further investigation to determine the *in vivo* consequences of transfusing these residual, small volume platelet products. In the interim, it is recommended to closely monitor volumes of LR-ADP after multiple aliquots are removed for patient transfusion and consider discarding LR-ADP once residual volumes are below 50 ml on day 4 or 5 of storage.

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Table 1

Metabolic characteristics of residual volumes of LR-ADP following aliquot removal for patient transfusion over 5 days of storage

	Days Post-Collection	
	Day 4 (n=22)	Day 5 (n=45)
Volume(ml)	61.6 ± 13.6 (30-90)	61.9± 11.1 (30-90)
pH	7.48±0.1 (7.27-7.62)	7.42± 0.1 (7.13-7.65)
pCO ₂ (mmHg)	13.8± 3.2 (9.3-20)	13.4 ± 4.5 (7.4-34.8)
pO ₂ (mmHg)	123.9± 9.6 (102-145)	123.3 ± 10.4 (85-138)
Bicarbonate(mmol/L)	10.1 ± 1.7 (6.7-13.6)	8.7 ± 2.7 (2.5-15.9)
Lactate (mg/dl)	86.2± 15.0 (43.7-106.8)	104.8± 19.2(72-157.5)

Table 2

Mean Platelet Counts ($\times 10^3/\mu\text{l}$) of LR-ADP aliquot products from an *in vitro* model of platelet storage

LR-ADP Aliquots	Day2	Day3	Day4	Day5
10-20ML (n=8)	1153	972	1028(975-1078)	977(844-1155)
30-40ML (n=12)	1153	1054(959-1198)	1076 (993-1286)	1005 (867-1098)
50-60ML (n=16)	1314	1072(959-1198)	1065 (958-1188)	1010(916-1178)
70-80ML (n=16)	1123	1081(934-1362)	1098(872-1429)	972(840-1214)

Table 3Metabolic characteristics of LR-ADP aliquot products from an *in vitro* model of platelet storage

	Day 2	Day 3	Day 4	Day 5
pH	7.37	7.33±0.11	7.31 ±0.17	7.05±0.19
pC02 (mmHg)	22.8	21.2 ± 14.5	8±2.4	6.2 ± 1.2
pO2 (mmHg)	116	121.9 ± 14.1	146.9± 12.6	147.3± 12.4
Bicarbonate(mmol/L)	13	9.9± 5.1	5.1 ± 2.6	2.2 ± 1.3
Lactate (mg/dl)	60.7	92.6± 38.2	139.8±28.9	169.8± 15.7
pH	7.37	7.39±0.08	7.48±0.12	7.35±0.18
pC02 (mmHg)	22.8	22.4±9.1	11.8±2.7	9.2± 2.4
pO2 (mmHg)	116	112.9± 18.8	142.5 ± 12.8	143.6± 8.1
Bicarbonate(mmol/L)	13	12.9± 3.9	9.4 ± 4	5.6± 3.1
Lactate (mg/dl)	60.7	76± 22.8	110.1±22.1	142.8± 18.4
pH	7.33	7.36±0.1	7.52±0.1	7.18±0.1
pC02 (mmHg)	23.9	26± 9.2	13.2± 3.0	12.6± 1.8
pO2(mmHg)	110	101.3 ± 19.6	135.1± 10.6	139.6± 12.6
Bicarbonate(mmol/L)	12.7	13.8±2.6	10.9±2.8	9.4± 1.6
Lactate (mg/dl)	51.2	66.5±7.5	90.7± 8	114.7±7.9
pH	7.21	7.31±0.1	7.42±0.1	7.44±0.1
pC02 (mmHg)	28.7	26.3±7.4	15.6±2.5	14.3± 1.9
pO2(mmHg)	103	103.1± 14.7	130.9± 12.3	135.3 ± 9.1
Bicarbonate(mmol/L)	11.3	13.2± 3.4	10.6± 3.7	9.8± 1.9
Lactate (mg/dl)	69.1	72.8± 14.2	94.5± 17.8	108.4 ± 11.5

Table 4

Platelet function of LR-ADP aliquot products from an *in vitro* model of platelet storage

	%Maximum Aggregation ADP/Collagen				% Aggregation Difference from Baseline*
	Day 2	Day 3	Day 4	Day 5	
10-20 ML (n=8)	83.8%	79.9%(76.8-89.1)	56.6%(42.2-66.5)	44.5%(15.9-70.1)	35.8%
30-40 ML (n=12)	83.8%	76.2%(48.3-100.3)	74.3%(44.7-101.7)	60.6%(34.4-79.7)	25.1%
50-60 ML (n=16)	93.1%	87.8%(74.1-100.3)	83.3%(67.1-78.3)	72.7%(67.1-78.3)	13%
70-80 ML (n=16)	99.6%	87.7%(74.1-102.6)	83.7%(67.9-103.7)	77.2%(67.5-88.4)	10.1%

* Baseline platelet aggregation studies were performed on the full volume LR-ADP received on day 2 or 3 post collection and prior to aliquot preparation. The percent change reflects the difference in aggregation from baseline to day 5 of storage.