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Effect of pneumatic tubing system transport on platelet apheresis units

Jevgenia Zilberman-Rudenko^{1,2,*,\$}, Frank Z. Zhao^{1,*}, Stephanie E. Reitsma², Annachiara Mitrugno², Jiaqing Pang², Joseph J. Shatzel³, Beth Rick¹, Christina Tyrrell⁴, Wohaib Hasan⁴, Owen J.T. McCarty^{2,\$}, and Martin A. Schreiber^{1,\$}

¹Division of Trauma, Critical Care and Acute Care Surgery, Department of Surgery, Oregon Health & Science University, Portland, OR

²Department of Biomedical Engineering, School of Medicine, Oregon Health & Science University, Portland, OR

³Division of Hematology & Medical Oncology, Department of Medicine, Oregon Health & Science University, Portland, OR

⁴Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR

Abstract

Purpose—Platelet apheresis units are transfused into patients to mitigate or prevent bleeding. In a hospital, platelet apheresis units are transported from the transfusion service to the healthcare teams via two methods: a pneumatic tubing system (PTS) or ambulatory transport. Whether PTS transport affects the activity and utility of platelet apheresis units is unclear.

Methods—We quantified the gravitational forces and transport time associated with PTS and ambulatory transport within our hospital. Washed platelets and supernatants were prepared from platelet apheresis units prior to transport as well as following ambulatory or PTS transport. For each group, we compared resting and agonist-induced platelet activity and platelet aggregate formation on collagen or von Willebrand factor (VWF) under shear, platelet VWF-receptor expression and VWF multimer levels.

Results—Subjection of platelet apheresis units to rapid acceleration/deceleration forces during PTS transport did not pre-activate platelets or their ability to activate in response to platelet agonists as compared to ambulatory transport. Platelets within platelet apheresis units transported

^{\$}Correspondence & Reprints: Jevgenia Zilberman-Rudenko, Department of Biomedical Engineering, Oregon Health & Science University, 3303 SW Bond Ave., Portland, OR 97239, Tel: 503-418-9307; Fax: 503-418-9311; zilberma@ohsu.edu.

^{*}J. Zilberman-Rudenko and F. Zhao are co-first authors.

^{\$}O. J.T. McCarty and M. A. Schreiber are co-senior authors.

Compliance with Ethical Standards:

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Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was received from all human participants. This article does not contain any studies with animals performed by any of the authors.

via PTS retained their ability to adhere to surfaces of VWF and collagen under shear, although platelet aggregation on collagen and VWF was diminished as compared to ambulatory transport. VWF multimer levels and platelet GPIb receptor expression was unaffected by PTS transport as compared to ambulatory transport.

Conclusions—Subjection of platelet apheresis units to PTS transport did not significantly affect the baseline or agonist-induced levels of platelet activation as compared to ambulatory transport. Our case study suggests that PTS transport may not significantly affect the hemostatic potential of platelets within platelet apheresis units.

Keywords

platelet apheresis units; platelet function; hemodynamics; hemostasis; pneumatic tubing system

INTRODUCTION

Upon vessel injury, exposed extracellular matrix (ECM) proteins, such as fibrillar collagen, trigger a series of events that lead to the formation of a hemostatic plug to staunch blood loss.[1, 2] The process of hemostasis depends on platelets tethering to the ECM in the presence of shear, leading to platelet adhesion, rapid cellular activation and accumulation of additional platelets.[3] ECM-bound von Willebrand factor (VWF) plays a critical role in initial platelet deposition via shear-dependent platelet receptor glycoprotein (GP) Ib binding to VWF.[4–6] Platelet receptors GPVI and $\alpha_2\beta_1$ mediate platelet activation leading to the release of secondary mediators and subsequent platelet aggregation. Platelet aggregation is enabled by platelet integrin $\alpha_{IIb}\beta_3$ and fibrinogen.[7]

Platelet apheresis units are used for effective management and stabilization of patients with thrombocytopenia and clinically significant bleeding associated with a high morbidity and mortality. In the United States, platelet apheresis units are most commonly collected by apheresis involving gentle centrifugation over a period of several hours from healthy adult volunteers. Guidelines have been established to ensure platelet apheresis unit quality including storage duration, temperature and handling prior to release of this therapeutic blood product for patient transfusion.[8] However, once in the hospital setting, there are no universal or strict guidelines for platelet apheresis unit delivery to care teams.

Pneumatic tubing systems (PTS) are widely used in hospitals to enable rapid and convenient transport of clinical samples, blood and therapeutic products, including platelet apheresis units, within hospitals. Interestingly, the use of PTS has been discouraged for transport of clinical samples intended for platelet function testing.[9, 10] Several studies have suggested that PTS transport of samples may affect the results of platelet functional tests including whole blood optical and electrode platelet aggregometry, [11–14] multiple parameters of rotational thromboelastometry (ROTEM)[13, 15] and closure time within sheared platelet function test (PFA-100).[12, 16, 17] It is not clear however what effect if any PTS transport may have on the activity or function of platelets in platelet apheresis units. The aim of this study was to determine whether exposure of platelet apheresis units to changes in gravitational forces during PTS transport affects baseline platelet activity or response to agonists as compared to ambulatory transport of platelet apheresis units.

MATERIALS AND METHODS

Materials and Reagents

Cross-linked collagen-related protein/CRP-XL was purchased from University of Cambridge (Cambridge, England). Thromboxane A₂ analog/U46619 and PAR-1 agonist/TRAP6 were from Tocris (Bristol, England). Epinephrine and fibrillar collagen were from Chrono-Log (Havertown, PA). Human VWF was from Haematologic Technologies, Inc (Essex Junction, VT). Fibrinogen was from Enzyme Research Laboratories (South Bend, IN). PPACK, rabbit anti-human anti-VWF primary antibody and goat anti-human anti-Vinculin primary antibodies were from Santa Cruz (Dallas, TX). Anti-CD41-PE, anti-CD62P-APC and Cytofix^{BD} were from BD Pharmingen (Franklin Lakes, NJ). Anti-CD31-eFluor450 was from eBioscience (San Diego, CA). Mouse anti-human anti-GPIIb/IIIa primary antibody was from GeneTex (Irvine, CA) and anti-mouse IgG -AF640 secondary was from Invitrogen (Carlsbad, CA). Seakem gold agarose was from Lonza (Basel, Switzerland). Recombinant rh-ADAMTS13 was from R&D Systems (Minneapolis, MN). SuperSignal West Dura Substrate was from Thermo Scientific (Waltham, MA). Other reagents were purchased from Sigma (St. Louis, MO).

Procurement and handling of platelet apheresis units

Single donor-per-unit platelet apheresis units were isolated by the American Red Cross, Pacific Northwest Blood Services Region, using a continuous-flow centrifugal apheresis machine Amicus (Fenwal Inc, Lake Zurich, IL), which utilizes a dual-stage separation technique with centrifugal force and belt-like geometrical configuration of separation and collection chambers (Table 1).[18] Each donor underwent a bilateral venipuncture allowing a simultaneous withdrawal of whole blood into sodium citrate, processing of blood within an apheresis machine and reinfusion of returning blood products. Within an apheresis machine, citrated whole blood was spun down at 1600rpm for an average of 10 minutes at RT to selectively isolate platelet rich plasma (PRP); red blood and white blood cells were reinfused back into the donor. PRP was then furthermore leuko-reduced by filtration and concentrated at 1600rpm for 10 minutes to remove a portion of the platelet-poor-plasma. Platelet apheresis units had an average concentration of $7.6 \pm 0.5 \times 10^5$ plts/ μ L, n = 20. Platelet apheresis units in apheresis bags were then received and stored by the OHSU Transfusion Medicine Services department. In accordance with USFDA guideline, platelet apheresis units were taken out of the clinical inventory 5 days post-collection and released for research use. Platelet apheresis units were collected from four ABO blood type donor groups: 7 from A, 7 from B, 3 from AB and 3 from O blood type; 4 platelet apheresis units were from Rh- and 16 PC units were from Rh+ donors.

On the day of an experiment, platelet apheresis unit was handled according to the flow chart (Fig. 1). Prior to transport, a basal sample was extracted from an apheresis bag containing a platelet apheresis unit with a 15 Gauge needle and a 20mL syringe at the OHSU Transfusion Service and stored in a 15mL polypropylene conical Falcon® tube in the lab. Subsequently, the remainder of the platelet apheresis unit was split into two apheresis bags and transported to the OHSU Trauma and Surgical Intensive Care Unit via two parallel methods: pneumatic tubing system (PTS; Swisslog TransLogic, Apeldoorn, The Netherlands) or ambulatory

(AMB) transport. Acceleration/deceleration forces generated during these transport methods were assessed using an internal iPod touch, IOS 9, accelerometer and recorded using a SensorLog v1.8 mobile app. Prior to insertion of the platelet apheresis units, an iPod was secured to the transport capsule as shown in Supp. Fig. 1. For reference, the centrifugal forces experienced during a 1600rpm spin in a GPKR centrifuge outfitted with rotor SH 3.7 S/N 576 (Beckman Coulter, Inc, Brea, CA; Table 2 & Fig. 2) were also quantified.

Human whole blood collection and preparation of concentrated platelet rich plasma and serum

To prepare fresh platelet-rich plasma (PRP), 40mL of human venous blood was drawn by venipuncture from healthy adult volunteers into 60mL syringe containing 1:10–3.8% sodium citrate in accordance with the OHSU Institutional Review Board. Whole blood was then transferred into a 50mL polypropylene conical Falcon® tube and spun down at 1600rpm for 10 minutes at RT; the acceleration/deceleration profiles were recorded using an accelerometer as described above. After the first spin, PRP was transferred into new 50mL conical tube and spun down again at 1600rpm for 10 minutes; the plasma volume was adjusted to achieve a final concentration of 4×10^5 plts/ μ L in concentrated (c)PRP (Table 1). The final yield was about 5mL of cPRP per 40mL whole blood donation.

Fresh serum was prepared by collection of human venous blood by venipuncture into a dry syringe. Non-anticoagulated blood was then left on a bench and allowed to clot for 30 minutes at room temperature. Serum was isolated by centrifugation at 2500rpm for 20 minutes in a Hermle Z300 centrifuge outfitted with rotor 221.12 V01 (Labnet, Edison, NJ).

Preparation of washed platelets and supernatants from platelet apheresis units or fresh cPRP

Platelet apheresis units or freshly prepared cPRP were divided into separate polypropylene tubes for select experimental procedures run in parallel. Prostacyclin (PGI_2 ; 0.1 $\mu\text{g/mL}$ final) and acid/citrate/dextrose (ACD; 1:10 final volume ratio) were added to 200 μL of platelet apheresis units or fresh cPRP prior to pelleting to generate washed platelets. Samples were then spun down twice at 2500rpm for 10 minutes to pellet the platelets; the final pellet was resuspended in 200 μL of serum, counted and adjusted to a final count of 4×10^5 plts/ μL . Washed platelets were allowed to rest for 45 minutes before being evaluated for baseline and agonist-induced activation and microaggregation. To purify platelet apheresis unit or cPRP supernatants, 20 μL of platelet apheresis units or cPRP were transferred into 1.7mL graduated copolymer polypropylene microcentrifuge and spun down at 2500rpm for 10 minutes.

Platelet activation and microaggregation assay

Platelet apheresis units or freshly prepared cPRP were pelleted and resuspended in human serum to a final concentration of 4×10^5 plts/ μL . Samples were then added to tubes containing vehicle buffer, ADP (3, 10 and 200 μM final), U46619 (1 and 10 μM final), a combination of ADP and U46619 (10 μM and 10 μM final each), CRP-XL (0.3, 1 and 10 $\mu\text{g/mL}$ final), TRAP6 (10 and 30 μM final), a combination of CRP-XL and TRAP6 (10 μM and 30 μM final each), 10 μM epinephrine, or 100 $\mu\text{g/mL}$ fibrillar collagen in the absence or

presence of 10 μ M ADP or 10 μ M epinephrine and incubated for 30 minutes at RT on a shaker agitated at 200rpm. Reactions were stopped by diluting samples 1:10 with a quenching solution consisting of modified HEPES-Tyroses with 40 μ M PPACK, 1.5% w/v Na-citrate (1:1, vol/vol).[19] Following this quenching step, 10 μ L of each sample was added to an additional 10 μ L of quenching solution containing the following anti-platelet antibodies: anti-CD41-PE, anti-CD62P-APC and anti-CD31-e450, to a final dilution of 1:50, and incubated for 30 minutes at RT in the dark. Samples were fixed by diluting 1:10 with quenching solution containing 12.5% Cytotfix^{BD}. 10,000 single platelets events were collected by quantifying a PE-conjugated platelet marker CD41 and the characteristic forward- and side-scatter scatter patterns using a fluorescence-activated cell sorter, FACS (Canto II; BD Biosciences). Platelet activation (%) was determined as the ratio of CD62P positive population of CD31/CD41 double-positive events as described previously.[19, 20] Percent microaggregate formation was determined by the upshift in fluorescence intensity in CD31/CD41 double-positive events. To measure platelet GPIba levels, platelets were incubated with 1:50 dilution of mouse anti-human monoclonal AK2 primary antibody for 30 minutes at RT followed by 30 minutes incubation with 1:50 final dilution of anti-CD41-PE and anti-CD31-eFluor450 and 1:1000 final dilution of anti-mouse IgG-AF640 secondary antibody.

Flow chamber assay

Glass capillary tubes/chambers (0.2 $\times$ 2 $\times$ 200 mm; VitroCom) were coated with fibrillar collagen (100 μ g/mL), VWF (100 μ g/mL) or fibrinogen (100 μ g/mL) for 1 hr at RT. Surfaces were blocked with 5 mg/mL denatured bovine serum albumin (BSA) for 1 hr at RT prior to assembly into a flow system as described previously.[19] Platelets content within platelet apheresis unit samples was quantified. Final platelet counts were adjusted to 4 $\times$ 10⁵ plts/ μ L using autologous supernatants. Platelet apheresis units were then perfused through the chambers for 10 minutes at an initial wall shear rate (300 s⁻¹). Platelet aggregation was visualized with differential interference contrast (DIC) microscopy and quantified using FlowJo software.

Immunoblot determination of VWF

50 μ g of plasma protein was loaded per well and run under non-reducing conditions as previously described.[21] A 1.5% low-to-medium resolution agarose gel was cast using Seakem gold agarose. Human VWF, incubated with or without rh-ADAMTS13, was used to confirm antibody specificity. After transfer to PVDF membrane, blots were incubated with an anti-VWF primary antibody followed by an HRP-conjugated secondary antibody. As a loading control, blots were probed for the high molecular weight housekeeping protein vinculin (117 kDa). Protein bands were visualized by chemiluminescence following incubation with SuperSignal West Dura Substrate. Quantitation of band area was performed with ImageJ (NIH).

Statistics

Data are shown as means $\pm$ SEM. Statistical significance of differences between means was determined by ANOVA. If means were shown to be significantly different, multiple

comparisons were performed by the Tukey test. Probability values of $P < 0.05$ were selected to be statistically significant.

RESULTS

Physical parameters of platelet apheresis unit handling

To evaluate the physical parameters experienced by platelets in platelet apheresis units during transport within a hospital, an iPod with the internal accelerometer was introduced into the transport capsule (Supp. Fig. 1) prior to insertion of the platelet apheresis units. After collection of a basal sample, platelet apheresis units were split into two transport study arms (Fig. 1) inserted into the transport capsules and the gravitational forces and transit time experienced by platelet apheresis units transported within the hospital at OHSU via PTS or AMB transport were examined. Platelet apheresis units were transported via PTS transport for 144 ± 8 seconds, including passage through a redistribution center and experienced a maximum of 7.96 ± 0.08 , 6.79 ± 0.37 and 7.87 ± 0.12 G forces ($n = 16$) in x-, y- and z-coordinate directions, respectively. Samples transported via ambulatory (AMB) transport were transported for 90 ± 2 seconds and experienced a maximum of 0.64 ± 0.10 , 0.46 ± 0.08 and 1.77 ± 0.09 G forces ($n = 16$) in x-, y- and z-coordinate directions, respectively. Notably, platelet apheresis units transported via PTS experienced frequent jolts of acceleration/deceleration amounting to 547 ± 77 transitions in the z-coordinate direction, whilst AMB-transported platelets traveled steadily without z-coordinate transitions (Table 2 & Fig. 2). Platelet apheresis units were subject to a similar degree of acceleration/deceleration transitions in the x- and y-coordinate directions during either PTS or AMB transport. As a comparator, platelets were subjected to a sustained acceleration of 8.16 ± 0.01 G forces ($n = 5$) in the z-coordinate direction during centrifugation at 1600 rpm with a smooth singular transition to baseline at the 'low' centrifuge brake setting (Table 2 & Fig. 2).

Effect of transport on platelet activation and aggregation

We first designed experiments to determine whether platelet apheresis unit transport had an effect on platelet activation or microaggregate formation. Washed platelets were isolated from platelet apheresis units or freshly prepared concentrated (c)PRP (Table 1), and washed platelet activation was assessed by quantifying P-selectin (CD62P) expression (Fig. 3A & Supp. Fig. 2A). Platelet microaggregate formation was quantified by measuring a shift in mean fluorescence intensity of double-positive CD31 and CD41 events (Fig. 3B & Supp. Fig. 2B) as described previously.[19, 20]

Consistent with previous reports, washed platelets purified from freshly prepared cPRP expressed significant levels of P-selectin from α -granules in response to increasing concentrations of the GPCR agonists (TRAP6, ADP, thromboxane A_2 analog U46619, epinephrine, or combinations of ADP and U46619), the ITAM-mediated (CRP-XL, fibrillar collagen) agonists, or combinations of these as compared to the vehicle control (Supp. Fig. 2A). The percent CD62P-positive washed platelets increased up to 4-fold in response to ADP, up to 12-fold in the presence of the TxA_2 analog, 13-fold in the presence of the combination of ADP and U46619, up to 15-fold in the presence of CRP-XL or TRAP6, 12-fold in the presence collagen alone, 30-fold in the presence of the combination of collagen

and either ADP or epinephrine, and 3-fold in the presence epinephrine alone as compared to baseline. Similarly, freshly prepared washed platelets formed microaggregates in response to increasing concentrations and combinations of agonists as compared to the vehicle control (Supp. Fig. 2B). The degree of platelet microaggregation increased up to 6-fold in response to ADP, 10-fold in response to U46619, 11-fold in response to the combination of ADP and U46619, up to 12-fold in response to CRP-XL alone or in combination with TRAP6, up to 9-fold in response to TRAP6 alone, at least 11-fold in response to collagen alone or in combination with ADP or epinephrine, or 5-fold in response to epinephrine alone.

Notably, centrifugation of whole blood and cPRP during preparation of freshly washed platelets did not induce significant baseline platelet activation nor microaggregate formation. In contrast, platelets purified from platelet apheresis units exhibited increased baseline platelet activation (Fig. 3A) and microaggregate formation (Fig. 3B) prior to either PTS or AMB transport. Furthermore, platelets isolated from platelet apheresis units were refractory to low doses of the secondary mediators of platelet activation, ADP and U46619 at baseline. The percent platelet activation increased only 2-fold in response to 200 μ M ADP or 10 μ M U46619, while only a 3-fold increase was observed in response to a combination of 10 μ M ADP and 10 μ M U46619. The platelet agonists collagen or epinephrine alone failed to induce activation of washed platelets from platelet apheresis units at baseline, while a 3-fold increase in CD62P expression was observed in response to the combination of collagen with ADP or epinephrine. Platelet activation reached a maximum of a 4-fold increase with increasing concentrations of CRP-XL, TRAP6 or combinations of CRP-XL and TRAP6. Similarly, the percent of platelet microaggregation increased only 2-fold in response to 200 μ M ADP, 7-fold in response to combination of U46619 and ADP, up to 8-fold in response to CRP-XL, 5-fold in response to TRAP6, 10-fold in response to the combination of CRP-XL and TRAP6 and up to 5-fold in response to the combinations of collagen and either ADP or epinephrine. We did not observe a statistically significant difference in platelet activation or microaggregate formation after transport via PTS or AMB as compared to baseline for any of the agonists. Our data suggest that transport of platelet apheresis units via either PTS or AMB routes does not affect agonist-induced platelet activation.

Effect of platelet apheresis unit supernatant on freshly prepared platelet activation and aggregation

Upon activation, platelets release secondary mediators including ADP to promote platelet activation via both paracrine and autocrine signaling. To test if supernatants from platelet apheresis units contain secondary mediators which promote platelet activation, fresh washed platelets purified from cPRP were incubated with increasing levels of supernatants from platelet apheresis units. Our data show that the presence of as low as 1% v/v platelet apheresis unit supernatant was sufficient to induce an increase in P-selectin expression and microaggregate formation in freshly washed platelets at baseline (Fig. 4A&B). The transport of platelet apheresis units via either PTS or AMB routes did not affect the ability of platelet apheresis unit supernatant to induce activation of freshly washed platelets. In contrast, the supernatant from cPRP failed to elicit a response from freshly washed platelets, suggesting that the observed effects of the platelet apheresis units on platelet reactivity was due to platelet apheresis unit storage rather than the supernatant isolation method used herein.

Together this data suggests that the supernatant from platelet apheresis units may promote a baseline level of activation, which may dampen further agonist-induced platelet reactivity. This is in line with previous studies showing that ADP-stimulated platelets tend to become refractory to stimuli over time.[22, 23]

Effect of platelet apheresis unit handling on platelet binding to collagen and VWF under shear

We next examined the effect of the transport of platelet apheresis units on the platelet hemostatic function of binding to exposed ECM and adhesive proteins under shear. For each transport method, final platelet counts were adjusted to 4×10^5 plts/ μ L using autologous supernatants. Platelet suspensions were perfused over immobilized fibrillar collagen, VWF or fibrinogen at a venous shear rate of 300 s^{-1} . We compared the degree of platelet adhesion (expressed as surface coverage) after 10 minutes. Our data show that platelets in platelet apheresis units prior to transport bound and aggregated on surfaces of collagen, VWF and fibrinogen (Fig. 5A&B). Equivalent levels of platelet adhesion and aggregation were observed for platelets in platelet apheresis units after AMB transport. Surprisingly, the degree of platelet adhesion and aggregation on collagen and VWF was reduced for platelets in platelet apheresis units that had been transported via PTS. This raised the question of whether PTS transport effected the expression of the platelet VWF receptor, GPIb, in light of the fact that platelet activation was unaffected by transport of platelet apheresis units via PTS.

Effect of platelet apheresis unit handling on levels of platelet GPIb receptor and VWF forms

We next studied whether transport of platelet apheresis units effected platelet GPIb receptor expression levels. Our results show that the expression level of GPIb was equivalent on platelets in platelet apheresis units prior to and after transport via either PTS or AMB routes (Fig. 6A). Moreover, GPIb levels on platelets in platelet apheresis units were equivalent to GPIb levels measured on freshly washed platelets.

We next examined levels of VWF multimer forms in supernatants isolated from platelet apheresis units prior to and following transport via either PTS or AMB routes. We found that when normalized to the housekeeping protein, vinculin, platelet apheresis unit fractions isolated at baseline or following PTS or AMB transport contained similar levels of total VWF, although the levels were slightly higher than the levels observed in freshly prepared platelet-rich plasma (Fig. 6B). The distribution of dimer and multimer forms of VWF was equivalent in the plasma of platelet apheresis units prior to transport as compared to following transport via either PTS or AMB routes (Fig. 6C). In summary, our data show that transport of platelet apheresis units via either PTS or AMB methods does not affect either platelet GPIb receptor expression or VWF multimer distribution as compared to prior to transport.

DISCUSSION

Hemorrhage remains a major cause of death in trauma patients.[24–27] Inclusion of platelet apheresis units as part of the early resuscitation strategy has been shown to promote survival of severely injured patients[28–30] and improve outcomes in patients with clinically relevant thrombocytopenia.[31] Platelet apheresis units are thus frequently rushed to the patient care teams as soon as the need is determined; commonly, pneumatic tubing systems (PTS) are used to accelerate and secure access of this vital transfusion product. Interestingly, several studies have indicated that transport of whole blood via PTS leads to abnormal platelet function test results as compared to AMB transport.[11–17, 32] In fact, these collective findings have led to establishment of the international recommendation against the use of PTS for transport of clinical samples for platelet function testing.[9, 10] It is puzzling that while PTS is no longer recommended for transport of clinical samples for platelet testing it is permitted and is frequently used for transport of platelet apheresis units to be transfused into patients. Our case study was designed to examine whether transport of platelet apheresis units via PTS transport effected platelet activation and function.

Donation of platelet apheresis units involves a significant donor time involvement as well as a number of short-term and long-term risks to donor including compromise of immediate hematologic parameters, thrombopoiesis and bone demineralization.[33, 34] Most of the studies looking at possible effects of PTS transport on platelet function have focused on the transport of whole blood sample via PTS and the use of a single concentration of a singular agonist or treatment coupled with platelet function tests.[35, 36] In our study we quantified the acceleration/deceleration profiles that platelet apheresis units experience within the OHSU PTS as compared to during ambulatory (AMB) transport. Our case study shows that subjecting platelet apheresis units to rapid changes in gravitational forces during PTS transport does not affect platelet response to soluble platelet agonists, GPIb receptor expression or VWF multimer levels. A slight decrease was observed in the degree of platelet aggregation on collagen and VWF, and we are currently investigating the mechanism behind this reduction in aggregate formation. It is unclear whether a slight decrease in aggregate formation is indicative of a clinically relevant loss of hemostatic function, however.

Our data indicate that platelets transported via PTS did not exhibit higher levels of activation or microaggregation as compared to platelets transported by an AMB method. This finding was consistent with Javela *et al.* who also showed that storage time rather than handling of platelet apheresis units promoted baseline platelet CD62P-secretion.[37] Other groups have also shown that PTS had no effect on platelet metabolic activity, activation or secretion as a function of time of storage.[32, 38, 39] Moreover, recent work by Kelly *et al.*, showed that the ability of platelet apheresis units to increase platelet count in non-bleeding cancer patients with thrombocytopenia was independent of the degree of platelet function as measured by aggregometry, P-selectin expression and fibrinogen binding.[40] Thus, perhaps as long as patients have a threshold concentration of functional platelets in circulation, the transfusion of additional platelets, even though they may exhibit reduced absolute activity, may be sufficient to stop bleeding. In this setting, the reactivity of the platelets within platelet apheresis units may be secondary to the ability to rapidly transport platelet apheresis units to patients at risk of hemorrhage. Conversely, in severely injured patients with

thrombocytopenia and platelet dysfunction, the transfusion of platelet apheresis units that retain platelet function may be paramount to promote hemostasis. Further studies are needed to properly assess the effect of transport on the stored platelet apheresis units function in different patient populations to guide an appropriate utilization of this important therapeutic reagent.

Clinical assessment of platelet function is complicated by a number of variables; historically, platelet function tests are notorious for their technical complexity and limited utility due to potential effects of sample handling during transport, platelet fragility and use of purification steps.[13, 35, 41–44] The assessment of platelet apheresis units is further complicated by platelet storage conditions during which platelets are prone to secrete secondary mediators, alter receptor availability, skew platelet response to stimuli and their ability to interact with certain ECM proteins.[22, 23, 39, 45–49] Furthermore, others have found that storage combined with increased frequency of the PTS transport dramatically decreases platelet function as assessed by aggregometry in the presence of collagen, ADP, TRAP or arachidonic acid.[39] During the past two decades, significant strides have been made to improve and simplify platelet testing including taking it out of the niche testing labs and making tests more portable and predictive of clinical outcomes.[35, 36] The potential commercialization of closed and open system methods using small volume whole blood samples hold the potential for the timely assessment of patient platelet function which may accelerate and simplify our understanding of patient-specific hemostatic capacity to guide potential interventions.[13, 50–56]

This report focused on the study of the effect of platelet apheresis unit handling during transport within a single hospital and a specific route. An important limitation to the generalizability of our study is the potential for variance between different PTS installations. The heterogeneity of hospital construction and layout dictates that the distances, transit time, and peak acceleration cargo experiences will vary somewhat from hospital to hospital, and even from floor to floor within hospitals, based on the specific layout and the specific pneumatic tube transport system installation. Within our own hospital for instance, blood products are transported to different floors of the hospital via PTS, likely producing variations in the forces the cargo experiences. Several prior publications encompassing different hospital systems have measured peak accelerations of approximately 8g, [57] to 15 g, [58], with peak accelerations of up to 25g having been reported in a study that evaluating higher speeds not routinely used for blood product transport (7 m/s).[15] This variance may explain some of the heterogeneity in reports of the effects of PTS on blood products.[59] Some authors have suggested hospitals perform internal evaluations of their PTS with g-loggers to determine the extent of forces that blood products undergo in transit.[59]

It is important to point out that the literature on the effects of PTS on platelet function and not universally consistent. Prior analysis of whole blood samples have found detectable differences in platelet aggregometry[11, 12, 14] and PFA-100 parameters in samples sent through PTS vs alternative handling procedures, [12] while other studies have not found an effect of PTS transport on platelet aggregometry.[16] There are multiple variables which may account for these differences including variation in the forces the platelets experience in each centers respective PTS system, [59] the fact that the cited studies evaluated whole

blood and the fact that the sources of whole blood vary from healthy volunteers, to patients receiving antiplatelet agents, to patients who may be critically ill. Some studies also sent the samples through the PTS unit multiple times, [13] or used PTS speeds not routinely used for blood product transport.[15] Clinically, the majority of previous studies evaluating whole blood are more generalizable to the accuracy of various platelet function assays performed on direct patient samples than to platelet transfusion, which was the main aim of our study.

We utilized platelet apheresis units released from the clinical inventory in accordance with the American Red Cross guidelines. The timeframe for platelet apheresis unit storage and expiration timeframe is set by the Red Cross to primarily prevent bacterial burden within blood component stored at RT. However, it is also likely that recently expired platelets maybe be less desirable for transfusion due to the accumulation of significant levels of platelet secondary mediators within the platelet apheresis unit supernatant.[60] Furthermore, our data is in accord with the notion that platelet apheresis unit storage may promote increased levels of high molecular weight VWF multimers (HMWM), which may be caused by stored platelet release of HMWM resistant to degradation by ADAMTS13.[61–64] With these limitations in mind it is important to note that we did not observe a difference in GPIb receptor expression between fresh and stored platelets.[65–70] While platelet transport via PTS had no effect on GPIb or VWF levels, we found that stored platelets exposed to high frequency of acceleration/deceleration jolts exhibited reduced aggregate formation on immobilized surfaces of collagen or VWF under shear rate of 300 s^{-1} . Future work using freshly isolated platelets will be focused on determining whether PTS plays a role in altering conformations or potentially causing shedding of platelet receptors (including GPIb and GPVI) and attempting to correlate these findings to clinical outcomes in patients who undergo platelet transfusion.[71, 72]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

PTS	pneumatic tubing system
AMB	ambulatory transport
PRP	platelet rich plasma

cPRP	concentrated platelet rich plasma
VWF	von Willebrand factor
GPIb	glycoprotein Ib
CD62P	P-selectin
ECM	extracellular matrix

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Figure 1. A flow chart of an experimental platelet apheresis unit handling procedure

A flow chart describing events on the day of an experiment. Prior to transport, a basal sample was extracted from an apheresis bag containing a platelet apheresis unit.

Subsequently, the remainder of the platelet apheresis unit was split into two apheresis bags and transported to the OHSU Trauma and Surgical Intensive Care Unit (ICU) via two parallel methods: pneumatic tubing system (PTS; Swisslog TransLogic, Apeldoorn, The Netherlands) or ambulatory (AMB) transport. All samples were subsequently processed at the research lab. When appropriate washed platelet and supernatants were purified from samples.

Figure 2. Physical parameters of platelet apheresis unit handling

Acceleration/deceleration profiles generated during transport via pneumatic tubing system (**A**; $n = 16$), ambulatory transport (**B**; $n = 16$) or platelet centrifugation (**C**; $n = 5$) as measured using an accelerometer. Representative z-coordinate tracings are shown. Data are reported as mean $\pm$ SEM.

Figure 3. Effect of transport on platelet activation and aggregation

Washed platelets isolated from platelet apheresis units were collected before (basal) or following transport via pneumatic tubing system transport (PTS) or by human courier, ambulatory transport (AMB). Samples were incubated with indicated agonists for 30 minutes, immunostained and evaluated by fluorescence-activated cell sorting (FACS) cytometry for percent platelet activation (CD41+/CD31+/CD62P+ events; **A**) or platelet microaggregate formation (high fluorescence intensity CD41+/CD31+ events; **B**). Mean $\pm$ SEM, n = 7.

Figure 4. Effect of platelet apheresis unit supernatant on fresh platelet activation and aggregation

Platelet apheresis units were collected before (basal) or following transport via pneumatic tubing system transport (PTS) or by human courier, ambulatory transport (AMB) and pelleted by centrifugation to isolate supernatants. Freshly prepared washed platelets were resuspended in serum supplemented with indicated fraction (percent total volume) of platelet apheresis unit supernatants. As a control, freshly washed platelets were resuspended in serum supplemented with supernatants isolated from cPRP. Fresh platelet activation (**A**) and microaggregate formation (**B**) in the presence of indicated levels of platelet supernatants were quantified by FACS; Mean±SEM, n = 4.

Figure 5. Effect of platelet apheresis unit handling on platelet binding to collagen and VWF under shear

Platelet apheresis units were collected before (basal) or following transport via pneumatic tubing system transport (PTS) or by human courier, ambulatory transport (AMB). Platelets content within platelet apheresis units was quantified and final platelet counts were adjusted to 4×10^5 plts/ μ L using autologous supernatants. Samples were perfused over surfaces of immobilized collagen, VWF or fibrinogen at a shear rate of 300 s^{-1} for 10 minutes.

Differential interference contrast (DIC) microscopy of platelet adhesion and aggregation representative of three separate experiments (A) and surface area quantification mean $\pm$ SEM (B) are shown. *, **, # and ## indicate statistically different groups with corresponding p-values of 0.010, 0.013, 0.027 and 0.003, respectively.

Figure 6. Effect of platelet apheresis unit handling on levels of platelet GPIb receptor and VWF forms

Platelet apheresis units were collected before (basal) or following transport via pneumatic tubing system transport (PTS) or by human courier, ambulatory transport (AMB) and were immunostained for surface expression of GPIb and evaluated by FACS. Geometric mean fluorescent intensity (GeomFI) of GPIb levels were normalized to levels found in freshly prepared cPRP samples. Mean $\pm$ SEM, n = 4. (A). In select experiments, platelet apheresis units and cPRP samples were pelleted by centrifugation to isolate and test supernatants for VWF multimers by Western blot. Total levels of VWF forms were normalized to vinculin (B; ns = not statistically significant with p = 0.1173, ** p = 0.0331 and *** p = 0.0123; n = 4). Ratios of VWF forms, high molecular weight multimer (HMWM), dimer and mature, were normalized to mature VWF forms (C; n = 4).

Table 1

Description of platelet preparations used in the study.

In text	Blood drawn by	Platelet concentrate preparation	Intended use	Stored at
<i>Platelet apheresis unit</i>	American Red Cross	a continuous-flow centrifugal apheresis machine	Health care, clinical	OHSU Transfusion Service
<i>Concentrated platelet rich plasma, cPRP</i>	OHSU Research Phlebotomist	Serial bench centrifugation	Research only	Used within 2 hours from blood draw

Table 2**Physical parameters of platelet apheresis unit handling**

Acceleration/deceleration forces generated during each transport type and platelet centrifugation were measured using an accelerometer (Apple iPod touch 16gb, 6th gen., IOS 9, SensorLog v1.8). Frequency and duration per jolt of acceleration/deceleration transition points were quantified.

	Gs generated			Acceleration/Deceleration jolts		
	Max	±	SEM	n	freq.	± SEM t (s)/jolt ± SEM
<i>Pneumatic tubing system transport</i>	x	7.96	± 0.08	16	269	± 54 1.0 ± 0.2
	y	6.79	± 0.37	16	205	± 26 1.1 ± 0.3
	z	7.87	± 0.12	16	547	± 77 0.4 ± 0.1
<i>Ambulatory transport</i>	x	0.64	± 0.10	16	164	± 49 17.5 ± 7.1
	y	0.46	± 0.08	16	257	± 106 6.8 ± 3.4
	z	1.77	± 0.09	16	1	± - 90.2 ± 1.7
<i>Centrifugation 1600 rpm</i>	x	7.57	± 0.01	5	2	± - 40.2 ± 2.6
	y	7.99	± 0.01	5	2	± - 30.2 ± 3.4
	z	8.16	± 0.01	5	2	± - 37.0 ± 2.3