The effects of pneumatic tube transport on fresh and stored platelets in additive solution

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Background. Limited scientific work has been conducted on potential *in vitro* effects of transport on pneumatic tube systems on blood components, in particular platelets.

Materials and methods. To evaluate the possible effects of the Swisslog TranspoNet system on the cellular, metabolic, phenotypic and secreting properties of fresh and stored platelets, we set up a four-arm paired study comparing transported and non-transported platelets. Platelets were aliquoted, prepared with the OrbiSac system and suspended in 70% SSP+ (n=8). All *in vitro* parameters were monitored over a 7-day storage period.

Results. Throughout storage, no differences were observed in glucose consumption, lactate production, pH, pCO_2, ATP, hypotonic shock response reactivity, CD62P, PAC-1, platelet endothelial cell adhesion molecule-1 or CD42b. The release of sCD40L increased ($p<0.01$) in all units but without any significant differences between groups.

Conclusion. The storage stability of all platelets conveyed by the Swisslog TranspoNet system was not impaired throughout 7 days of storage. The Swisslog TranspoNet system does not, therefore, seem to be a risk for increased metabolic activity, activation or release reactions from the platelets. This lack of effect of the pneumatic tube transport system did not seem to be affected by the age of the platelets or repeated transport.

Keywords: platelets, platelet additive solution, pneumatic tube system.

Introduction

Different pneumatic tube transport systems (PTS) have been widely used for the transport of specimens to the laboratory and these systems have been reported both to have no effect or adverse negative effects[1-6]. However, surprisingly little research has been conducted on the effects of PTS on blood components[7,8], in particular platelets[9]. The impact of different PTS on platelets stored in additive solution seems to be completely unexplored. There is, therefore, still no consensus about the advisability of using PTS to transport blood components, especially platelet units stored in additive solution.

One of the key properties of platelets is their capacity to respond quickly to different kind of rheological conditions, become activated and secrete factors that promote blood clotting and tissue regeneration[10]. Platelets can, therefore, be easily triggered when they encounter an artificial environment. Production and storage of platelets represents no exception to this notion[11-13]. The extent to which the manipulation involved in these processes compromises platelet function *in vivo* is not known, but it is nevertheless important to characterise aberrant situations in which the ability of platelets to withstand storage may be affected. One such potential situation may be the acceleration and deceleration forces during transport in a PTS which may not only have an influence on specimens[1,2], but also on platelets. On the one hand transport systems may exacerbate factors contributing to existing morphological, biochemical or functional derangements caused by processing and storage[12,14] and may, therefore, be associated with an increased risk of decreased post-transfusion survival of platelets[15-17]. On the other hand, an opportunity to send platelets stored in additive solution with a PTS would increase the availability and efficiency in meeting the demand for such products.

To evaluate the possible effects of PTS on cellular, metabolic, phenotypic and secreting properties of fresh and stored platelets, we set up a four-arm paired study comparing transported and non-transported platelets suspended in 70% SSP+. All parameters were followed of over a 7-day storage period.

Materials and methods

Preparation and storage of platelets

In this study, we carried out paired experiments in four arms comparing *in vitro* effects of a PTS on fresh

and stored platelets. The fourth arm was included to study the potential harmful effects of doubling the transport time.

Platelets were collected on day 0 from normal blood donors who met standard donation criteria and gave written, informed consent according to institutional guidelines. Four hundred and fifty millilitres of whole blood were drawn into either a CPD/SAG-M quadruple-bag blood container system (Fenwal, La Châtre, France) or the NPT 6280LE blood bag system (MacoPharma, Mouvaux, France). After storage at room temperature for 2-6 hours, the whole blood units were centrifuged (2,700 *g*) for 10 minutes at 22 °C. Automatic equipment was used for the preparation of blood components (Optipress, Fenwal or Macopress Smart, MacoPharma), including buffy coats. Buffy coats (20 units) were stored overnight and combined in a large-volume container to create an ABO-identical primary pool selected on the basis of the platelet concentration in donor blood. In total, 8 ABO-identical primary pools were created from 160 buffy coat units. The primary pools were split into 4 equal parts for the preparation of platelet units.

All 4 buffy coat units were prepared on day 1 using the OrbiSac system (TerumoBCT, Zaventem, Belgium)[18] to yield platelet units stored in approximately 30% plasma and 70% SSP+ (MacoPharma, Mouvaux, France). The maximum g-force generated by the OrbiSac system is 1,196 *g*. To avoid disintegration and adverse negative effects on the platelets[19], air and foam were excluded from all units immediately after preparation. All units were then stored on a flat bed agitator (60 cycles a minute; model PC900i, Helmer, Noblesville, IN, USA) in a temperature-controlled cabinet at 22±2 °C. The samples were drawn aseptically on days 2, 5 and 7 for subsequent transport by the PTS.

Transport of platelets with the Swisslog TranspoNet system

The Swisslog TranspoNet system (Westerstede, Germany) was used for transport of the test units. The TranspoNet system is a PC-controlled air tube system with numerous functions. The Windows-based software supports the system configuration, operation and monitoring. In our study, the one way transport of the platelet units lasted between 2 and 3 minutes and was performed under randomly controlled room temperature conditions. The rate of the cartridges is 0-5/6 m per second. Cartridges are slowed by air and the system is designed for smooth acceleration/deceleration. All tested platelet units were immediately returned, prior to analysis, which means a maximum transport time of 6 minutes in each case.

The runs with the Swisslog TranspoNet system were performed according to the following scheme: (A) Reference (no run); (B) Swisslog Transponet system run on day 2; (C) Swisslog Transponet system run on day 7; and (D) Swisslog Transponet system run on days 2 and 7.

Analysis of cellular, metabolic, *in vitro* functional, phenotypic and secretion markers

In vitro cellular parameters, including platelet counts (10^9/L and 10^9/unit) and mean platelet volume, were determined using CA 620 Cellguard (Boule Medical, Stockholm, Sweden). The volume (mL) was calculated by weighing the contents of the storage bag, in grams, on a scale (Mettler PB 2000, Mettler-Toledo, Albstadt, Switzerland) and dividing the result, in grams, by 1.01 (1.01 g/mL is the density of the storage medium composed of approximately 70% SSP+ and 30% plasma).

The extracellular metabolic environment was studied using routine blood-gas equipment (ABL 800, Radiometer, Copenhagen, Denmark). The parameters determined included (37 °C), pCO_2 pO_2 (kPa at 37 °C), glucose (mmol/L) and lactate (mmol/L). Bicarbonate (mmol/L) was calculated based on the other variables measured. The pH of all samples was measured at 37 °C. Rosenthal's factor of 0.0147 unit/1 °C was, therefore, used to correct pH to the temperature of sampling (22 °C). This factor gives an approximation of the change in pH of the sample per degree centigrade when it is warmed anaerobically from the collecting temperature of 22 °C to 37 °C.

According to Bertolini and Murphy[20], the assessment of swirling was scored as 0, 1 and 2. The white blood cell count on day 1 was determined with a Nageotte chamber and a microscope (Zeiss, Standard, Chester, VA, USA)[21]. Hypotonic shock response reactivity (HSR) as well as the extent of shape change (ESC) measurements was performed using a dedicated microprocessor-based instrument (SPA 2000, Chronolog, Havertown, PA, USA) with the modifications of these tests described by VandenBroeke *et al*[22]. The total adenosine triphosphate (ATP) concentration, (µmol/10^{11} platelets) was determined with a Luminometer (Orion Microplate Luminometer, Berthold Detection Systems GmbH, Pforzheim, Germany) on the basis of principles described by Lundin[23].

The extracellular lactate dehydrogenase (LDH) activity (% of total), a marker of cell disintegration, was measured with a spectrophotometric method (Sigma Aldrich kit 063K6003, St Louis, MO, USA; Spectrophotometer Jenway 6500, Staffordshire, UK)[24]. The levels of expression of PAC-1, a marker of cellular responsiveness towards ADP, CD62P a marker of activation, CD42b a marker of adhesive capability and platelet endothelial cell adhesion molecule (PECAM-1) were determined by staining, as described in previous

studies[13,25,26]. sCD40L concentrations were determined with commercial enzyme-linked immunosorbent assays (Quantikine, CD40 Ligand Immunoassay DCDL40) in accordance with the manufacturer's (R&D Systems Inc, Minneapolis, USA) recommendations. All measurements were performed in duplicate on an HT3 Microtiter Plate Reader (Anthos Labtec Instruments GmbH, Salzburg, Austria) at 466 nm and the results for the sCD40L concentrations are given in pg/mL.

Detection of bacteria

Bacterial contamination was assessed on day 7 using the eBDS system. This system indicates the presence of bacteria through a decrease in oxygen tension, as measured in platelet samples after incubation for 24 hours at 35 °C[27].

Statistical analyses

The results are given as means (of eight values) and standard deviations unless otherwise indicated. A repeated measure ANOVA including a *post hoc* test with Bonferroni's adjustment was performed. Four different groups were studied over time (days). "Days" was the repeated factor and "Group" was a between factor. The P value represents the differences between groups at specific time points or differences within groups between day 2 and day 7. In both cases the values were considered statistically significant at $p<0.01$. The analyses were carried out using Statistica software, version 9 StatSoft, Inc 1984-2007 (SPSS, Chicago, IL, USA).

Results

The platelet and leucocyte counts and content on day 2 are given in Table I. The cellular, metabolic and *in vitro* functional parameters are all listed in Tables II and III. The phenotypic and secretory properties of the platelets are presented in Table IV.

Table I - Platelet count and content of the different platelet units on day 2*.

Platelet units	Volume (mL)	Platelet count (10^9/L)	Platelet content (10^9/unit)	Leucocyte content (10^6/unit)
A. Reference (no run)	382±6	918±64	350±20	<0.2
B. Swisslog Transponet system run on day 2	383±8	877±60	336±20	<0.2
C. Swisslog Transponet system run on day 7	387±57	890±71	344±25	<0.2
D. Swisslog Transponet system run on days 2 and 7	387±6	885±49	342±15	<0.2

* Results are expressed as mean±SD (n=8)

Table II - The cellular and metabolic in vitro effects on platelets of transport with a PTS (Swisslog Transponet system) after various days of storage.

Parameter	Day		
	2	5	7
Mean platelet volume (fL)			
A Reference (no run)	8.5±0.3	8.6±0.2	8.6±0.2
B PTS run day 2	8.4±0.2	8.6±0.2	8.5±0.2
C PTS run day 7	8.4±0.2	8.5±0.1	8.5±0.2
D PTS runs day 2 and 7	8.4±0.1	8.4±0.2	8.5±0.2
Lactate dehydrogenase (%)			
A Reference (no run)	3.4±1.2	4.9±1.5	6.4±1.7
B PTS run day 2	4.4±2.5	4.3±1.2	4.4±0.5
C PTS run day 7	4.4±1.5	4.9±1.6	4.6±0.8
D PTS runs day 2 and 7	4.2±1.0	4.2±1.6	4.8±1.4
Glucose (mmol/L)			
A Reference (no run)	4.7±0.5	3.5±0.5	2.5±0.5**
B PTS run day 2	4.7±0.5	3.5±0.5	2.5±0.5**
C PTS run day 7	4.7±0.4	3.6±0.5	2.6±0.5**
D PTS run day 2 and 7	4.7±0.5	3.5±0.5	2.5±0.5**
Lactate (mmol/L)			
A Reference (no run)	5.9±0.7	7.9±0.9	10.1±0.9**
B PTS run day 2	5.9±0.5	8.0±0.4	9.9±0.6**
C PTS run day 7	5.9±0.6	8.0±0.6	9.9±0.8**
D PTS run day 2 and 7	5.8±0.6	8.1±0.5	10.0±0.7**
pH (22 °C)			
A Reference (no run)	7.243±0.022	7.328±0.021	7.336±0.028**
B PTS run day 2	7.251±0.021	7.327±0.023	7.331±0.032**
C PTS run day 7	7.253±0.021	7.329±0.022	7.335±0.034**
D PTS runs day 2 and 7	7.245±0.022	7.324±0.023	7.333±0.032**

Values are reported as mean±standard deviation (n=8). Statistically significant differences within groups between day 2 and day 7 are marked ** ($p<0.01$).

No significant difference in platelet counts or contents was detected between the groups throughout the storage period (data not shown). No differences were observed in mean platelet volume or extracellular lactate dehydrogenase activity as a percentage of total, which remained stable at low levels in all reference and transported platelet units. No statistically differences were observed in glucose/lactate concentration, glucose consumption rate (0.49±0.05 µmol/10^9 platelets/day or lactate production rate 0.60±0.01 µmol/10^9 platelets/day between any of the units compared.

Throughout storage, no differences were observed in pH, partial pressures of oxygen (data not shown) and carbon dioxide (pCO_2), calculated bicarbonate and ATP levels, Subsequently, HSR reactivity, response to ESC and CD62P expression showed similar trends without

Table III - Metabolic and *in vitro* functional effects on platelets of transport with a PTS (Swisslog Transponet system) after various days of storage.

Parameter	Day		
	2	5	7
pCO2 (kPa at 37 °C)			
A Reference (no run)	2.98±0.20	2.13±0.17	1.94±0.1**
B PTS run day 2	2.89±0.3	2.14±0.2	1.95±0.2**
C PTS run day 7	2.92±0.26	2.19±0.23	2.0±0.2**
D PTS runs day 2 and 7	2.95±0.22	2.20±0.17	1.94±0.2**
Bicarbonate (mmol//L)			
A Reference (no run)	5.5±0.4	4.8±0.5	4.5±0.6**
B PTS run day 2	5.5±0.6	4.8±0.6	4.5±0.7**
C PTS run day 7	5.5±0.5	5.0±0.7	4.6±0.7**
D PTS runs day 2 and 7	5.5±0.5	5.0±0.6	4.6±0.6**
ATP (µmol/10^{11}platelets)			
A Reference (no run)	7.34±0.23	7.36±0.24	7.37±0.23
B PTS run day 2	7.76±0.31	7.67±0.37	7.51±0.55
C PTS run day 7	7.80±0.32	7.76±0.34	7.80±0.21
D PTS runs day 2 and 7	8.15±0.39	7.94±0.22	7.68±0.16
HSR (%)			
A Reference (no run)	69.8±8.5	66.2±9.2	62.7±5.5
B PTS run day 2	71.1±12.6	65.0±9.8	59.1±8.0
C PTS run day 7	71.8±11.4	64.4±9.6	61.9±7.9
D PTS runs day 2 and 7	71.7±11.9	64.9±9.5	56.9±10.0
ESC (%)			
A Reference (no run)	24.9±2.3	17.4±2.8	16.0±1.5**
B PTS run day 2	23.4±4.1	17.5±2.6	15.1±2.6**
C PTS run day 7	24.8±3.1	18.5±3.7	16.3±4.2**
D PTS runs day 2 and 7	25.1±1.0	19.8±2.8	17.4±2.4**

Values are reported as mean±standard deviation (n=8). Statistically significant differences within groups between day 2 and day 7 are marked ** ($p<0.01$).

Table IV - The phenotypic and secretory properties of platelets transported with a PTS (Swisslog Transponet system) after various days of storage.

Parameter	Day		
	2	5	7
CD62P (%)			
A Reference (no run)	20.98±2.87	23.59±2.80	28.02±2.54**
B PTS run day 2	21.58±2.99	24.75±2.73	28.80±2.32**
C PTS run day 7	20.05±3.63	25.87±1.27	29.20±2.03**
D PTS runs day 2 and 7	20.58±2.84	24.71±2.05	29.01±1.54**
PAC-1 (%)			
A Reference (no run)	39.97±5.16	31.80±5.25	24.58±6.42**
B PTS run day 2	37.14±4.79	28.35±3.03	22.20±6.10**
C PTS run day 7	35.71±2.80	28.92±3.42	21.81±6.33**
D PTS runs day 2 and 7	35.15±4.06	25.99±3.86	20.97±5.54**
CD42b (%)			
A Reference (no run)	99.02±0.5	98.53±0.76	97.79±1.03
B PTS run day 2	98.98±0.49	98.60±0.73	98.03±0.91
C PTS run day 7	99.06±0.46	98.64±0.67	98.03±0.83
D PTS runs day 2 and 7	99.03±0.36	98.58±0.74	97.81±0.86
PECAM-1 (%)			
A Reference (no run)	99.71±0.06	99.64±0.19	99.56±0.24
B PTS run day 2	99.32±0.75	99.0±1.63	99.42±0.41
C PTS run day 7	99.38±0.76	99.61±0.21	99.34±0.58
D PTS runs day 2 and 7	99.28±1.15	99.60±0.25	99.42±0.41
PECAM-1 (MFI)			
A Reference (no run)	12.7±0.9	12.9±0.8	15.7±5.1**
B PTS run day 2	12.2±1.4	13.8±1.0	15.8±4.9**
C PTS run day 7	12.5±1.3	13.0±0.7	15.1±4.4**
D PTS runs day 2 and 7	12.3±1.2	13.1±0.1	15.2±3.4**
sCD40L (pg/mL)			
A Reference (no run)	5,990±819	8,851±930	9,574±678**
B PTS run day 2	6,007±740	8,760±543	9,068±641**
C PTS run day 7	5,018±965	8,081±892	8,707±1003**
D PTS runs day 2 and 7	6,236±972	9,086±1301	9,719±886**

Values are reported as mean±standard deviation (n=8). Statistically significant differences within groups between day 2 and day 7 are marked ** ($p<0.01$).

any significant differences between the units. Similarly, no differences were observed in the expression of the conformational epitope on the GpIIb/IIIa, determined by using PAC-1, or in the expression of PECAM-1 at any time point between groups. CD42b decreased slightly during storage but without any significant differences between the units. Swirling remained at the highest level at all times in all reference and transported units. The release of sCD40L, a marker of storage lesion and ageing, increased statistically during storage in all units but without significant differences between the groups at any time point.

Generally, within specific groups, between day 2 and day 7 statistically verified differences in several markers of storage lesion and ageing were detected, including decreases in ESC, increases in CD62P expression, decreases in the response to PAC-1, up-regulation of the PECAM-1 epitope and increased release of sCD40L.

Bacterial contamination

Bacterial contamination was not detected in any of the units.

Discussion

This four-armed paired study describes the *in vitro* effects of the Swisslog TranspoNet system on fresh and stored platelets in additive solution and potential effects of repeated transport. The use of PTS to transport platelet units stored in additive solution would increase the availability and efficiency in meeting demands for these products.

In this study, none of the *in vitro* parameters selected to demonstrate different aspects of platelet function were statistically different between the four groups at any time point. Our data demonstrate lesion and ageing effects over time in all groups, but conveyance in the Swisslog TranspoNet system did not reinforce these negative changes. The capacity of the Swisslog TranspoNet system to act as a potential source of increased metabolic activity, activation and release reactions from the platelets does, therefore, seem to be insignificant. This fact seems not to be affected by the age of the platelets or repeated transport after adequate storage.

Our results did not show any difference between groups, but our statistically verified data over time within each group well reflect the lesion effect caused by the processing and storage of platelets. Although platelet metabolism appeared to have the ability to generate an equivalent concentration of ATP during storage[28], we observed that an increased degree of activation apparently affected the ability of platelets to respond to agonists during storage. This conclusion is based on generally increased expression of CD62P, related to impaired response to ESC and PAC-1, during storage. Although none of our markers indicated additional negative effects from PTS transport or doubling the transport time, supplementary markers reflecting cell response, relative to their activation state seem to provide contradictory information. Recently presented data indicated reduced sensitivity to TRAP-induced aggregation after repeated transport with PTS[9] which may be indicative of a general impairment of platelet functionality. There is currently little insight into the biological impact of these conflicting data and further studies are, therefore, warranted. One of the main issues regarding lesion effects is a clear understanding of reversible cellular changes versus irreversible changes, and how these changes are related to increased risk of decreased post-transfusion platelet survival[15-17]. Recently published data suggest that irreversible damage of stored platelets is attributable to the loss of mitochondrial membrane potential[26].

In our study, increased activation and, speculatively, up-regulation of PECAM-1 appeared to be associated with increased release of sCD40L. The relationship between increased activation and surface-expressed CD40L, cleaved and shed from the platelet surface in a time-dependent manner as sCD40L, is in line with data presented earlier[29,30]. Accumulation of sCD40L in stored blood components is not desirable as it may be associated with both transfusion-related acute lung injury (TRALI) and transfusion-related febrile reactions[30,31]. New technology in which immunomodulatory factors can be reduced from platelet units may, therefore, reduce adverse negative effects after transfusion.

The ability of PECAM-1 (CD31) to mediate cell-cell adhesion and up-regulate integrin function gives this molecule potentially significant roles in a variety of important processes[32]. Thus, potential storage effects[12,33,34] on this essential structure may be of importance as PECAM-1 is an efficient signaling molecule known to have diverse roles in platelet function[35]. One such potential effect that caught our attention was the suggestion that PECAM-1 signaling inhibits the aggregation of platelets[36]. However, any biological significance of the up-regulation of PECAM-1 during storage of platelets remains uncertain and merits further study.

In summary, our data clearly show that all parameters reflecting different aspects of platelet function remained largely unaffected by using the Swisslog TranspoNet system to transport platelets. We, therefore, conclude that fresh and stored platelets can be transported with the Swisslog TranspoNet system, which would probably increase the availability and efficiency of fulfilling the demand for these products.

The Authors declare no conflicts of interest.

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