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Platelet Additive Solutions SSP+ and T-PAS+ Are Interchangeable for Platelet Concentrate Storage despite Differences in Composition and Plasticizer

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Abstract

Introduction: Platelet additive solutions support ex vivo storage of platelet concentrates used for transfusion. The composition of platelet additive solutions within one generation (i.e., PAS-E) is similar but not identical. Additionally, the platelet additive solution storage bag may contain different plasticizers. This study compares the effect of two PAS-E solutions (SSP+ vs. T-PAS+, stored in a DEHP-containing and DEHP-free bag, respectively) to investigate if both additive solutions are interchangeable for platelet concentrate storage. ***Methods:*** Platelet concentrates stored in plasma supplemented with SSP+ or T-PAS+ were compared by using a pool-and-split design. Platelet metabolism was investigated using a blood gas analyzer. The degree of platelet storage lesion was determined by flow cytometry to measure granule release and phosphatidylserine scrambling. ***Results:*** The quality of platelet concentrates stored in either SSP+ or T-PAS+ is acceptable as pH decreased only slightly as a function of time. PH remained above 7.2 on exiration day +1 (day 6), which is far above the minimal criterion of 6.4. Platelet storage lesion was comparable between the two study groups with only limited α-granule release and phosphatidylserine surface expression in both groups after storage for 5 days, $p = 0.547$ and $p = 0.825$, respectively. ***Conclusion:*** This study supports a safe switch between SSP+ and T-PAS+ storage solutions for platelet concentrates despite slight differences in storage solution composition and DEHP content.

Introduction

Platelet additive solutions (PASs) were developed in the 1980s to improve storage conditions of platelet concentrates and to lower the risk for allergic and plasma-associated transfusion reactions like ABO hemolytic reactions and transfusion-related acute lung injury [1]. An additional advantage of PASs is the possibility to perform photochemical treatment for the inactivation of bacteria and other pathogens in platelet concentrates. PASs mitigate lesions caused by the photochemical treatment. For this, platelets are suspended in a solution with approximately 40% plasma and 60% PAS [2].

PASs are electrolyte solutions used to maintain the quality of the platelets during storage by adding specific nutrient ingredients [1]. PAS-B has the simplest composition containing only sodium chloride, sodium citrate, and sodium acetate. Later, phosphates were added to increase buffering capacity that generated PAS-C solutions. PAS-C also increased platelet glycolysis resulting in increased lactic acid production. To counteract this, a next generation of PAS (PAS-E) was developed containing potassium and magnesium ions [3].

Regulation (EC) No 1907/2006 by the European Parliament and the Council concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) bans the use of bis(2-ethylhexyl) phthalate

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(DEHP) from medical devices (>0.1% [w/w]). DEHP belongs to a family of chemicals called phthalates, which are added to plastics (including blood bags systems) to make them flexible. Concerns about leaching of DEHP in the blood products and the consequential negative health effect by endocrine system disruptions have resulted in this European legislation [4]. Platelet concentrates are currently already stored in DEHP-free storage bags. However, DEHP leaching into the final platelet product can still occur through the DEHP-containing collection bag, tubing, or PAS storage bags [5].

In this study, the in vitro characteristics of platelets prepared and stored in two different platelet storage solutions, i.e., SSP+ and T-PAS+ will be compared. Although both solutions have a similar composition (both categorized PAS-E), the raw chemical materials are slightly different. SSP+ contains disodium phosphate in an anhydrous form, while T-PAS+ contains disodium phosphate in a dodecahydrate form. This eventually results in a double phosphate concentration in T-PAS+ compared to SSP+, which might differentially impact platelet metabolism [6]. Additionally, SSP+ is stored in a DEHP-containing polyvinyl chloride (PVC) bag in our study, while the plastic storage bag for T-PAS+ is a DEHP-free polyolefin bag.

Methods

Study Design

The impact of T-PAS+ and SSP+ as platelet concentrate storage solutions was investigated in a pool-and-split design to obtain identical start products. See online supplementary Table 1 (for all online suppl. material, see https://doi.org/10.1159/000538003) for all details of the PASs. A jumbopool of 12 ABO compatible buffy coats, obtained from voluntary whole blood donations, was prepared before splitting into two equal volumes corresponding to six buffy coats. The paired buffy coat pools were supplemented with either 250 mL SSP+ (Macopharma, Tourcoing, France) or T-PAS+ (TerumoBCT, Lakewood, CO, USA) before platelet concentrate preparation as described before [7]. Briefly, the bag was centrifuged at 542 *g* for 450 s at 22°C to separate red and white blood cells from platelets. The buoyant platelet suspension was taken off by automated separation (Macopress Smart, Macopharma) while passing over a leukocyte reduction filter. The platelet concentrates were subjected to amotosalen photochemical treatment (Intercept, Cerus Corporation, Concord, CA, USA) for pathogen reduction [8]. Hereafter the platelet concentrates were stored at room temperature (22°C ± 2°C) in a temperature-controlled environment with continuous agitation. Samples for analysis (~2 mL) were taken at the start of platelet concentrate storage (day 2 [D2]), 72 h (D5), and 96 h later (D6) corresponding to expiration day or expiration day +1, respectively. Platelet concentrate shelf life is limited to 5 days in Belgium because hemovigilance data had shed doubt on transfusion efficacy after storage and pathogen inactivation [9]. In total, 17 paired platelet concentrates were compared in this study, using either T-PAS+ or SSP+ as platelet storage solution.

Swirling, Platelet Count, and Metabolic Parameters

Platelet concentrates were assessed visually for swirling, yielding an integral score between 0 and 3. A low swirling score indicates an aberrant platelet morphology and deteriorated quality. Platelet concentrates with a swirling score ≥2 are considered suitable for transfusion. Platelet counts were measured using an automated hematology analyzer (XN1000, Sysmex). Metabolic parameters were measured in a point-of-care blood gas analyzer (RAPIDPoint 500, Siemens Healthineers, Groot-Bijgaarden, Belgium).

Flow Cytometry

Expression of CD62P (phycoerythrin-anti-CD62P, Life Technologies, Carlsbad, CA, USA) and phosphatidylserine (BV421-Annexin V, Biolegend, San Diego, CA, USA) was analyzed with an acoustic focusing flow cytometer (Attune NXT, Life Technologies). Platelets were incubated with antibody or ligand for 10 min at room temperature in buffer with 10 mM HEPES-buffered saline with 1 mM $MgSO_4$. For Annexin V measurements, buffers were supplemented with 2 mM $CaCl_2$. The signals of the isotype antibody controls were used to set threshold gates including 0.5% of 10,000 negative events. For annexin V gating, samples were incubated with annexin V in the absence of ionized calcium. Median fluorescence intensities and percentage positive events were determined of 10,000 events in a platelet morphology gate, based on forward and side scatter properties.

Statistics

Sample means ± standard deviation were compared by paired *t* tests or by two-way ANOVA with multiple comparisons. Results were considered significant if p values <0.05. Statistical analysis was with Prism version 9.5.1 (GraphPad Software Inc.).

Results

Pool-and-Split Design

At the start of the study (D2), the composition of the paired platelet concentrates was comparable when looking at platelet content and total volume (shown in Table 1). The plasma content (%) was 38.69 ± 0.01 and 39.27 ± 0.01 for the SSP+ or T-PAS+ subgroup, respectively, which is within the plasma carryover acceptance criteria [32%; 47%] for pathogen reduction using the amotosalen photochemical method [8]. Products in both subgroups passed quality checks with an acceptable pH and a maximal swirling score (i.e., = 3) at the start of the study.

Platelet Metabolism

Platelet concentrate glucose levels decreased as a function of time at the same rate for platelet concentrates stored in SSP+ or T-PAS+ (shown in Fig. 1a). Simultaneously, lactic acid levels increased as a function of time without significant differences between both subgroups (shown in Fig. 1b). Hence, glucose consumption rates and lactic acid production rates were not significantly different between the subgroups ($p = 0.43$ and $p = 0.74$, respectively; shown

Table 1. Platelet concentrate content and quality control at start (D2)

	SSP+	T-PAS+	Statistics
Platelet concentrate volume, mL	329.8 (±7.7)	323.6 (±10.1)	$p = 0.07$
Platelet concentration, $\times 10^6$/μL	1.054 (±0.061)	1.070 (±0.088)	$p = 0.14$
Platelet content, $\times 10^{11}$	3.479 (±0.250)	3.480 (±0.321)	$p = 0.87$
Plasma content, %	38.69 (±0.01)	39.27 (±0.01)	$p = 0.06$
pH	7.27 (±0.03)	7.26 (±0.03)	$p = 0.23$
Swirl score	3	3	$p = 1.00$

Data are given as mean with standard deviation ($n = 17$). Statistical analysis was performed by two-tailed paired *t* tests.

in Fig. 1c, d). The pH of the platelet products was stable during the course of the study and none of the platelet concentrates had levels below 6.4, even on expiration day +1 (D6; shown in Fig. 1e).

Platelet Storage Lesion

All platelet concentrates met the criteria for transfusion, even at the day of expiration (D5) with a mean swirling score of 2.94 ± 0.24 and 3.00 ± 0.00 for SSP+ and T-PAS+ stored platelet concentrates, respectively. Both CD62P expression and annexin V binding, as surrogate markers for spontaneous α-granule release and phosphatidylserine expression, respectively, increased slightly as a function of storage time. The obtained values were not significantly different for the tested platelet storage solutions at all measured timepoints (shown in Fig. 2a, b).

Discussion

The short shelf life of platelets is a well-known challenge for blood banks. Minimizing the platelet storage lesion by using PASs tailored to the needs of platelet concentrate preparation and storage is important for maintaining platelet quality and ultimately a successful transfusion. PAS-E has been proven to be an acceptable substitute to plasma for platelet storage and resulted in decreased allergic reactions upon transfusion [10, 11].

The two PASs tested in this study are both of the PAS-E generation but are manufactured from different raw chemicals. SSP+ contains anhydrous disodium phosphate, while T-PAS+ contains the dodecahydrate form of disodium phosphate (shown in Table 1). Once resolved, the chemical composition of both solutions is nearly identical with only a difference in final phosphate concentration. Phosphates might have counteracting effects on platelet metabolism as they work both as a buffer to prevent a decrease in pH during storage but on the contrary also act as stimulants of platelet glycolysis to increase lactic acid production [12]. Although a different impact on platelet metabolism and storage lesion was not anticipated based on these slight differences, platelet characteristics were studied in vitro to investigate the impact when interchanging the investigative PAS-E solutions.

Platelets are metabolically very active cells and consume about 0.3 μmol glucose per 10^{10} platelets per hour [13]. The produced lactic acid would result in the acidification of the platelet concentrate resulting in a decrease of pH over time. However, due to the buffering capacities of the PASs, pH values were never below the acceptance criterion of 6.4 [1]. pH is generally used as a quality parameter for stored platelet concentrates, but there is some controversy regarding its suitability for this purpose, especially when 60–70% PAS is used [14]. Glucose consumption and lactic acid production could be better predictors of platelet metabolism. Both quality parameters were used to track platelet metabolism in this study and the obtained values were comparable for the investigated subgroups, as they were for pH.

Platelet storage lesion is a series of biochemical, structural, and functional changes to platelets during their time ex vivo. Following transfusion in a patient, this platelet storage lesion can translate into a lower platelet recovery or a shorter survival time, and ultimately a decreased ability to stop or prevent bleeding. Platelet storage lesion is the consequence of exposure to artificial surfaces (e.g., plastics) and high centrifugation forces during preparation causing platelet activation and subsequent release of biochemical proteins from platelet granules. Storage of platelet concentrates at room temperature can further result in altered platelet surface proteins like glycoproteins and phospholipids, which could lead to altered platelet functionality [15]. To investigate the differential effect of both PASs, the expression of both CD62P and phosphatidylserine as two well-described biological response modifiers and markers of platelet storage lesion was determined. There were no significant differences found between both PASs. It was

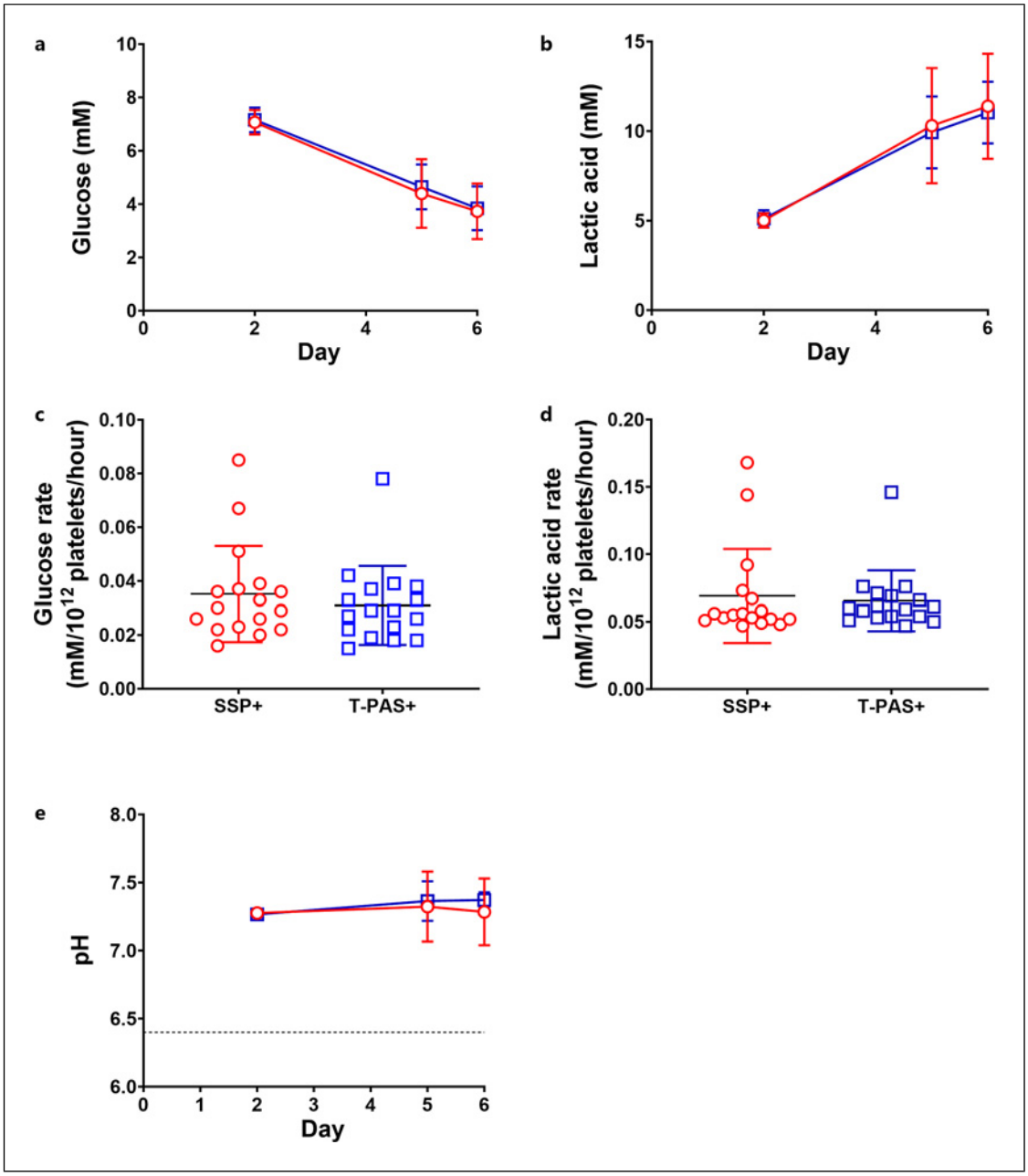

Fig. 1. Platelet metabolism. Glucose (**a**) and lactic acid (**b**) (mM) values in platelet concentrates at each time point measured. Glucose consumption (**c**) and lactic acid production rate per hour over 6 days of storage per 10^{12} platelets (**d**). **e** pH measured at T = 22°C. The dashed line shows the lower acceptance criterion for platelet concentrates. ○, platelets stored in SSP+; □, platelets stored in T-PAS+. Data are shown as mean ± SD (n = 17). Statistical analysis was performed by two-way ANOVA with multiple comparisons for **a**, **b**, and **e** and two-tailed paired t test for **c** and **d**.

beyond the scope of our study to define the complete activation status of the stored platelets as this has been described before (e.g., studying integrin $\alpha I_{Ib}\beta_3$ activation [7] or soluble release marker levels [sP-selectin, CD40L] [16]). When comparing platelet function at the day of production (D2) with the day of expiration (D5), a twofold increase in P-selectin (median fluorescent intensity) and a fourfold increase for phosphatidylserine (% positive cells) were detected, suggesting a small increase of platelet storage lesion. Increases of this order of magnitude were described before and are with an acceptable range for the storage period [17, 18].

From the 1960s on, platelets were stored in DEHP-plasticized (PVC) containers. The storage time for platelets in these containers was limited because of low oxygen

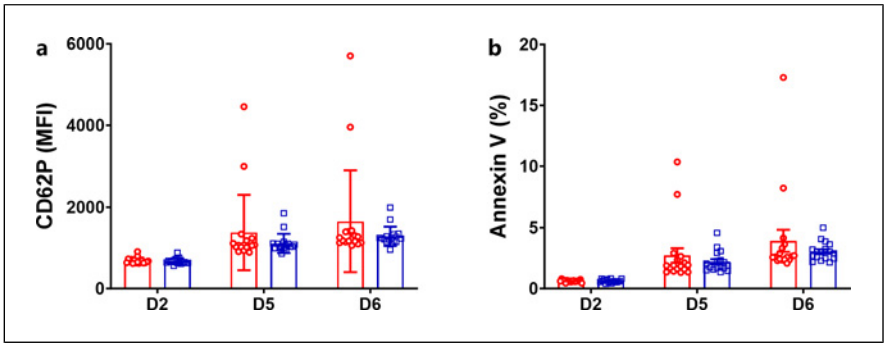

Fig. 2. Platelet storage lesion. **a** Median fluorescent intensity (MFI) for CD62P binding as determined by flow cytometry. **b** The percentage of platelets binding to Annexin V as measured by flow cytometry. Platelets stored in SSP+ (red bars); platelets stored in T-PAS+ (blue bars). Data are shown as mean ± SD. Statistical analysis was with two-way ANOVA with multiple comparisons.

permeability, carbon dioxide accumulation, and lactic acid production resulting in a decline in pH. An additional disadvantage of the containers is DEHP leaching in platelet concentrates. DEHP has been detected in platelet concentrates but is not suspected to have any effect on platelet functionality [19, 20]. PVC containers are now mostly replaced by gas-permeable plastics like polyolefin, which showed better outcomes on platelet storage and functionality, and which have the additional advantage of being DEHP-free [5]. However, other products in the platelet concentrate production chain like tubing, filter housing, or the PAS storage bag still contain DEHP as they are stored in the PVC storage bag. This remnant DEHP could potentially leach into the platelet concentrate product. In the light of the upcoming REACH legislation on medical devices, it will be essential to move to blood products that are completely DEHP-free.

This study demonstrated that platelet concentrates suspended in either T-PAS+ or SSP+ and treated with the INTERCEPT System resulted in equivalent platelet quality during storage in vitro and no impact of different PAS plastic storage bags (DEHP-containing or DEHP-free) could be detected. This result supports interchangeability for T-PAS+ and SSP+ for platelet concentrate storage despite slight differences in composition and plasticizer.

Acknowledgments

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Statement of Ethics

This study was in accordance with national regulations on the use of blood products for validation and/or quality control, which is exempt from a separate review by an Ethical Committee. All donors consented in writing to use their products for this study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

B.V.A. coordinated the study. B.V.A., B.S., H.B.F., and K.R.S. designed the study. S.V. and B.S. performed laboratory experiments and data collection. B.V.A., K.B., and K.R.S. performed data interpretation. B.V.A. and K.R.S. performed statistical analysis. B.V.A., K.B., and K.R.S. drafted the manuscript. H.B.F. and V.C. supervised the experimental activities and managed the laboratory. All authors edited and approved the final manuscript.

Data Availability Statement

All data generated and analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

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