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Evaluation of Different Preparation Procedures of Pathogen Reduction Technology(Mirasol®)-Treated Platelets Collected by Plateletpheresis

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Key Words
Pathogen inactivation · Pathogen reduction · Platelet concentrates · Mirasol® · Riboflavin

Summary
Background: The Mirasol® pathogen reduction technology (PRT) for platelet concentrates (PC) uses riboflavin and UV light (270–360 nm). We evaluated the impact of PRT on platelets in comparison to standard single-donor PC. **Material and Methods:** Platelets were resuspended in autologous plasma. After 2 h rest without agitation, PC were split into an untreated control unit (C-PC) and an immediately treated unit (T-PC) (series I). In series IV, split PC were stored under agitation over night before PRT was carried out. Platelet quality was assessed by pH, glucose consumption, lactate production rate, LDH, soluble sCD62p and CD62p expression with and without TRAP (thrombin receptor-activating peptide) over 7 days. **Results:** Series I: On day 5, pH values were lower for T-PC (6.8 ± 0.2 vs. 7.4 ± 0.1, C-PC), accompanied by a higher glucose consumption rate of 0.069 ± 0.016 vs. 0.035 ± 0.006 mmol/10^{12} platelets/h and lactate production rate of 0.126 ± 0.031 vs. 0.063 ± 0.011 mmol/10^{12} platelets/h. CD62p using TRAP was lower for T-PC (50 ± 11 vs. 62 ± 14%). Baseline activation was higher in T-PC (35 ± 12 vs. 28 ± 15%). Longer initial rest time had no impact on these results (series II/III/IV). **Conclusion:** PRT leads to an increase of platelet metabolism and activation independent of the length of the initial rest times. PC resuspended in autologous plasma should be stored at maximum up to day 5.

Schlüsselwörter
Pathogeninaktivierung · Pathogenreduktion · Thrombozytenkonzentrat · Mirasol® · Riboflavin

Zusammenfassung
Hintergrund: Das Mirasol®-Verfahren (PRT) unter Verwendung von Riboflavin und UV-Licht (270–360 nm) wurde für die Pathogeninaktivierung von Thrombozytenkonzentraten entwickelt. Wir haben den Einfluss des Verfahrens auf die Thrombozytenqualität im Vergleich zu Standard-Apherese-Thrombozytenkonzentraten untersucht. **Material und Methoden:** Thrombozyten wurden in autologem Plasma resuspendiert. Nach zweistündiger Lagerung ohne Agitation wurde das Thrombozytenkonzentrat in Serie I in ein unbehandeltes Kontrollprodukt (C-PC) und ein unmittelbar behandeltes Testprodukt (T-PC) aufgeteilt. In Serie IV wurden die geteilten Produkte vor Behandlung über Nacht unter Agitation gelagert. pH-Wert, Glukoseverbrauch, Laktatproduktion, LDH, lösliches sCD62p sowie die CD62p-Expression mit und ohne TRAP (Thrombinrezeptor aktivierende Peptide) wurden über 7 Tage Lagerung untersucht. **Ergebnisse:** Serie I: PRT führt zu einem pH-Abfall auf 6,8 ± 0,2 vs. 7,4 ± 0,1 (C-PC) an Tag 5. Parallel dazu kam es zu einem erhöhten Glukoseverbrauch von 0,069 ± 0,016 vs. 0,035 ± 0,006 mmol/10^{12} Thrombozyten/h sowie einer erhöhten Laktatproduktion von 0,126 ± 0,031 vs. 0,063 ± 0,011 mmol/10^{12} Thrombozyten/h (C-PC). Die maximale Thrombozytenaktivierbarkeit unter Verwendung von TRAP lag für T-PC niedriger (50 ± 11 vs. 62 ± 14%). Die Basisaktivierung bei diesen Präparaten war höher (35 ± 12 vs. 28 ± 15%). Eine verlängerte initiale Lagerungszeit hat keinen Einfluss auf die Ergebnisse (Serie II/III/IV). **Schlussfolgerung:** PRT führt zu gesteigertem Thrombozytenmetabolismus und Zellaktivierung unabhängig von der initialen Lagerungszeit. Thrombozyten, resuspendiert in autologem Plasma, können maximal bis Tag 5 gelagert werden.

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Introduction

To prevent transfusion-transmitted infections after transfusion of platelet concentrates (PC), different pathogen reduction strategies have been pursued: the photochemical inactivation procedure using amotosalen in combination with UVA light and the pathogen reduction technology (PRT) utilizing riboflavin (RB) in combination with UV light.

Upon application of UV light and absorption of low wavelength photons, the PRT treatment results in nucleic acid alterations directly. Moreover, UV light in the presence of RB causes the formation of 8-oxodGuo in nucleic acids. Formation of 8-oxodGuo may be mediated by the direct interaction of photoexcited RB with DNA, resulting in one-electron oxidation of the guanine moieties. RB enhances the rate of DNA modification and reduces the likelihood of DNA repair and reactivation of treated pathogens and white blood cells [1–3]. It could be demonstrated that the replication of different viruses, bacteria species [4], parasites [5], and leucocytes [6] is inhibited after PRT.

Considering the implementation of this technique into routine use, the question arises whether this pathogen reduction method has an influence on the quality and storability of the platelets. We collected single-donor platelets resuspended in autologous plasma and investigated the influence of PRT procedure on the in vitro quality of platelets in comparison to single-donor standard PC. We focused on platelet metabolism and activation over a storage period of 7 days, assessing different initial resting times before PRT treatment.

Material and Methods

In accordance with the German regulations for blood donation, single-donor PC were collected from regular blood donors using the Amicus™ cell separator (Fenwal Europe, Mont Saint Guibert, Belgium). Each donor gave informed consent to participate in the study. A double dose of 6.0×10^{11} (1,180–2,100 $\times 10^6$ cells/ml) platelets was harvested and resuspended in 450 ml autologous plasma using ACD-A as the anticoagulant.

- *Series I:* PC double dose units (n = 14) were stored for 2 h without agitation. Afterwards, each unit was split into 2 identical PC subunits of approximately 225 ml, followed immediately by PRT.
- *Series II/III:* PC double dose units (n = 6 each) split into 2 subunits after the initial storage of 2 h without agitation and stored for 4 h (series II) and for 6 h (series III) under agitation before PRT was carried out.
- *Series IV:* PC double dose units (n = 6) were split into 2 subunits after the initial storage of 2 h without agitation and stored over night with agitation. PRT was carried out the next day within 24 h of collection.

Test Platelet Units – Pathogen Reduction Technology

The test subunit (T-PC) was transferred to an illumination/storage bag (CaridianBCT, Lakewood, CO, USA). RB is packed in a disposable polyolefin bag to maintain appropriate solution stability through the sterilisation process and prior to use. It is wrapped in a foil pouch protecting the vitamin from ambient light. RB is connected to the PC using a sterile docking device. The mixture of PC and RB (35 ± 5 ml, 500 µmol/l RB in a 0.9% saline chloride, pH 4.0–5.0) leads to a final RB concentration of approximately 65–70 µmol/l. The Mirasol process has been validated for RB concentrations up to 160 µmol/l. For illumination, the PC was placed in a fix position in the illumination device and exposure to light took place (6.2 J/ml of UV light, 265–370 nm). During this process, the device agitated the PC and controlled the temperature of the product and the UV lamps' outputs. Depending on the volume of the PC, the illumination time was about 6–10 min.

As further bag transfer or adsorption of the resulting photoproducts is not required, the PC were stored on a platelet incubator under agitation (22 ± 2 °C) over a period of 7 days.

Control Platelet Units

The control subunit (C-PC) was transferred into the same storage bag as the T-PC (CaridianBCT). Autologous plasma (35 ml) was added to the corresponding T-PC at the same time as RB. Thus, we were able to directly compare both products due to identical storage container characteristics and volume. The C-PC was also stored in a platelet incubator under agitation for 7 days.

Laboratory Parameters

Using a sterile transfer tubing set connected to the storage bag, samples were withdrawn under laminar flow using a syringe. In series I/II/III, investigations for C-PC and T-PC were carried out on day 0 after addition of RB or plasma, and before PRT treatment as well as on days 1, 3, 5, and in the morning of day 8 with respect to a potential storage up to day 7. In series IV, sampling was carried out on day 1 after addition of RB or plasma, and before PRT, and on days 2, 4, 6, and 8.

The samples were assayed for platelet count, red blood cell (RBC) and white blood cell (WBC) counts (only series I), blood gas analysis, glucose consumption and lactate production rate, lactate dehydrogenase (LDH), soluble p-selectin (sCD62p) as well as surface-expressed p-selectin (CD62p) with and without activation using TRAP (thrombin receptor-activating peptide) and swirling.

Blood Gas Analysis and Cell Count

Samples were immediately analysed for pH (22 °C), HCO_3^-, pO_2, and pCO_2 with a blood gas analyser (Synthesis 10; Instrumentation Laboratory, Kirchheim, Germany). Platelet count was analysed automatically (CELL-DYN 3200; Abbott, Wiesbaden, Germany). RBC and WBC were analysed using FACS (Coulter Epics XL; MCL Beckmann Coulter, Krefeld, Germany).

Platelet Activation Markers

For estimation of baseline activation, CD62p expression (GMP140, p-selectin) was determined by flow cytometry using 2-color labelling with monoclonal antibodies. PC samples were adjusted to 25,000 platelets/µl with PBS. A 30 µl diluted PC sample was incubated with 10 µl of anti-CD41-PE and 20 µl of anti-CD62p-FITC for 5 min in the dark at room temperature. The reaction was stopped by adding 1 ml of PBS (pH 7.4, 4 °C). For the negative control, 20 µl of anti-IgG1-FITC was used instead of the anti-CD62p-FITC. 20,000 events were counted by the flow cytometre (FACSCalibur; Becton Dickinson, Heidelberg, Germany) and results were reported in percent positive within a range defined by the negative control. In a second PC sample, CD62p expression was measured after activation by 100 µmol/l TRAP. This analysis gives information on the maximal activation capacity of the platelets.

Levels of p-selectin in plasma (sCD62p) were quantified by an ELISA (Human sl-selectin; R & D System GmbH, Wiesbaden-Nordenstadt, Germany).

Evaluating cell dilatation or cell lysis due to platelet activation, LDH was quantified by automated methods (Dimension RxL; Siemens Medical System, Dade Behring, Eschborn, Germany) according to the manufacturer's recommendations.

Biochemical Studies

In the supernatant of the PC, glucose and lactate concentration were measured by automated methods (Dimension RxL) according to the manufacturer's recommendations.

Table 1. Series I (n = 14): preparation data

	Original bag	After addition of RB before illumination	Day 1	Day 3	Day 5	Day 8
Platelet yield, × 10^{11}						
T-PC	5.8 ± 0.7	2.8 ± 0.3*	2.4 ± 0.3*	2.3 ± 0.2*	2.2 ± 0.3*	2.1 ± 0.3*
C-PC	5.8 ± 0.7	2.9 ± 0.2	2.7 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2
Platelet content, × 10^3/µl						
T-PC	1,277 ± 159	1,099 ± 119*	1,048 ± 117*	1,064 ± 108*	1,101 ± 124	1,125 ± 143
C-PC	1,277 ± 159	1,147 ± 70	1,148 ± 89	1,133 ± 68	1,153 ± 93	1,201 ± 107
pH, 22 °C						
T-PC	7.4 ± 0.1	7.4 ± 0.1*	7.3 ± 0.1*	7.1 ± 0.1*	6.8 ± 0.2*	6.3 ± 0.0*
C-PC	7.4 ± 0.1	7.2 ± 0.3	7.4 ± 0.0	7.4 ± 0.1	7.4 ± 0.1	7.2 ± 0.2

*p < 0.05; T-PC (PRT-treated unit) versus C-PC (untreated control unit).

Table 2. Series IV (n = 6): preparation data

	Original bag	After addition of RB before illumination day 1	Day 2	Day 4	Day 6	Day 8
Platelet yield, × 10^{11}						
T-PC	5.8 ± 0.4	2.8 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.2 ± 0.1
C-PC	5.8 ± 0.4	2.7 ± 0.2	2.6 ± 0.2	2.4 ± 0.2	2.4 ± 0.3	2.3 ± 0.2
Platelet content, × 10^3/µl						
T-PC	1,364 ± 75	1,164 ± 48	1,048 ± 50	1,038 ± 48	1,046 ± 52	1,009 ± 67
C-PC	1,364 ± 75	1,094 ± 98	1,066 ± 97	1,029 ± 88	1,031 ± 114	1,008 ± 98
pH, 22 °C						
T-PC	7.2 ± 0.1	7.5 ± 0.1	7.3 ± 0.4*	7.1 ± 0.1*	6.8 ± 0.2*	6.3 ± 0.0*
C-PC	7.3 ± 0.1	7.5 ± 0.2	7.6 ± 0.1	7.5 ± 0.1	7.3 ± 0.1	7.2 ± 0.1

*p < 0.05; T-PC (PRT-treated unit) versus C-PC (untreated control unit).

Statistics

All data are expressed as mean ± 1 standard deviation (SD). The Wilcoxon paired t-test was used for comparison of test versus control units. To compare the data within 1 group over the storage period, the Friedman test was used.

Statistically significant differences were based on p-values < 0.05. Statistical analyses were performed with commercially available software for personal computers (SPSS for Windows XP; SPSS Software GmbH, Munich, Germany).

Results

Volume, Platelet Content, Red Blood Cells, White Blood Cells, Blood Gas Analysis

– *Series I:* On average, PC were resuspended in a collection volume of 455 ± 11 ml. After 2 h of rest without agitation, PC were split into 2 subunits with a volume of 223 ± 4 ml for T-PC and 223 ± 6 ml for C-PC. Due to sampling, the volume dropped during storage in both series.

On average, $5.8 \pm 0.7 \times 10^{11}$ platelets were collected. After splitting, T-PC contained $2.8 \pm 0.3 \times 10^{11}$ and C-PC $2.9 \pm 0.2 \times 10^{11}$ platelets per unit. Due to sampling, the content dropped until the end of the storage period (table 1).

WBC and RBC values were measured immediately afterwards. Results were comparable between T-PC and C-PC, with WBC counts of $0.05 \pm 0.001 \times 10^6$ per unit and RBC counts of $0.28 \pm 0.003 \times 10^9$ per unit.

The pH dropped significantly for T-PC from day 1 on. On day 5, T-PC showed values of 6.8 ± 0.2 in comparison to C-PC with values of 7.4 ± 0.1 (p = 0.001) (table 1).

This was paralleled by a drop of the HCO_3^- values from 17.3 ± 1.5 mmol/l (day 0) to 3.8 ± 1.3 mmol/l (day 5) to 0.7 ± 0.1 mmol/l (day 8) for the T-PC and from 22.4 ± 1.3 mmol/l (day 0) to 11.4 ± 1.5 mmol/l (day 5) to 6.7 ± 2.0 mmol/l (day 8) for the C-PC. Values of T-PC were overall significantly lower. But as the course of HCO_3^- was paralleled in both groups from day 0 to day 8, the differences resulted due to the addition of the 35 ml of plasma to the C-PC.

– *Series IV:* On average, PC were resuspended in a collection volume of 428 ± 10 ml. After splitting, C-PC and T-PC volume were about 216 ± 4 ml. On average, $5.8 \pm 0.4 \times 10^{11}$ platelets were collected. After splitting, T-PC contained $2.8 \pm 0.1 \times 10^{11}$ and C-PC $2.7 \pm 0.2 \times 10^{11}$ platelets (table 2).

The pH dropped significantly for T-PC from day 2 on. On day 6, T-PC showed values of 6.8 ± 0.2 in comparison to C-PC with values of 7.3 ± 0.1 (p = 0.018) (table 2).

Table 3. Series I (n = 14): laboratory findings

	After addition of RB before illumination	Day 1	Day 3	Day 5	Day 8
CD62p, %					
T-PC	24.7 ± 8.2*	22.8 ± 6.2*	30.2 ± 6.0*	35.2 ± 11.5*	41.7 ± 11.6*
C-PC	15.8 ± 5.2	16.2 ± 5.6	25.7 ± 8.3	28.5 ± 15.4	24.6 ± 9.7
CD62p + TRAP, %					
T-PC	65.8 ± 8.9	53.3 ± 13.3*	50.3 ± 10.6*	49.8 ± 10.8*	41.6 ± 10.8
C-PC	61.5 ± 8.7	63.8 ± 12.7	61.8 ± 13.0	62.5 ± 13.8	47.6 ± 13.3
LDH, IU/l					
T-PC	179 ± 80	133 ± 46*	147 ± 48*	167 ± 51*	200 ± 47
C-PC	154 ± 57	159 ± 56	169 ± 57	179 ± 57	188 ± 57
Glucose, mmol/10^{12} cells					
T-PC	12.7 ± 0.7*	12.5 ± 1.3*	8.8 ± 0.8*	5.0 ± 1.4*	0.4 ± 0.6*
C-PC	14.5 ± 0.7	14.0 ± 1.1	12.3 ± 0.8	10.3 ± 1.0	7.2 ± 1.3
Lactate, mmol/10^{12} cells					
T-PC	4.1 ± 1.4*	6.0 ± 1.3*	12.3 ± 2.5*	18.9 ± 4.2*	26.6 ± 3.8*
C-PC	3.5 ± 1.1	4.0 ± 1.0	7.7 ± 1.0	10.6 ± 1.6	14.5 ± 3.1

*p < 0.05; T-PC (PRT-treated unit) versus C-PC (untreated control unit).

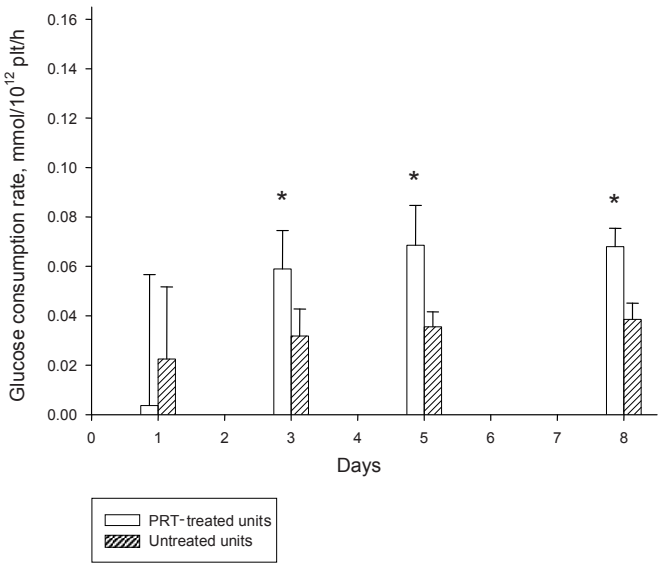

Fig. 1. Series I (n = 14): glucose consumption rate. *p < 0.05 PRT-treated units versus untreated units.

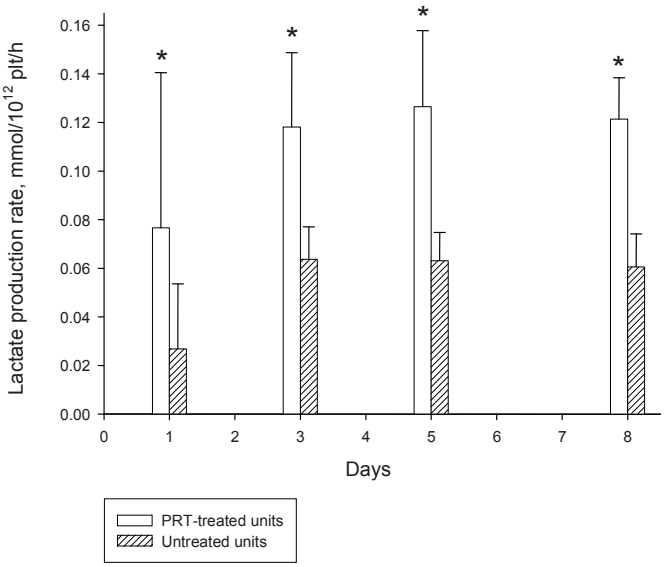

Fig. 2. Series I (n = 14): lactate production rate. *p < 0.05 PRT-treated units versus untreated units.

This was paralleled by a drop of the HCO_3^- values from 16.8 ± 2.0 mmol/l (day 1) to 4.8 ± 1.2 mmol/l (day 6) to 1.1 ± 0.1 mmol/l (day 8) for the T-PC and from 17.1 ± 1.8 mmol/l (day 1) to 11.0 ± 1.3 mmol/l (day 6) to 6.9 ± 1.6 mmol/l (day 8) for the C-PC. But as the course of HCO_3^- was paralleled in both groups from day 0 to day 8, the differences resulted due to the addition of the 35 ml of plasma to the C-PC.

Biochemical Measurements

Glucose and lactate were calculated in mmol/l per 10^{12} platelets. The rate of glucose consumption and lactate production was calculated as mmol/10^{12} platelets per hour.

– *Series I:* Glucose concentration per 10^{12} cells was generally higher in C-PC due to the addition of autologous plasma (table 3). Nevertheless, values indicate a higher consumption in T-PC. Lactate results per 10^{12} cells were generally lower in C-PC (table 3). The accumulation of lactate over time was higher in T-PC, indicating a higher production. This was confirmed by calculating the glucose consumption rate (fig. 1) and the lactate production rate (fig. 2) which were significantly higher for T-PC throughout the storage period.

– *Series IV:* Glucose concentration per 10^{12} cells was generally higher in C-PC (table 4) due to the addition of autologous

Table 4. Series IV (n = 6): laboratory findings

	After addition of RB before illumination day 1	Day 2	Day 4	Day 6	Day 8
CD62p, %					
T-PC	26.4 ± 16.7	27.5 ± 6.2*	45.9 ± 9.8*	45.4 ± 10.5	61.9 ± 9.6*
C-PC	19.5 ± 8.6	21.4 ± 6.7	24.2 ± 7.0	36.9 ± 16.8	26.8 ± 3.9
CD62p + TRAP, %					
T-PC	81.9 ± 7.3	82.0 ± 6.0*	72.8 ± 13.4*	61.1 ± 10.2*	66.1 ± 7.2
C-PC	81.1 ± 5.7	85.2 ± 4.7	80.9 ± 7.6	71.1 ± 10.8	71.1 ± 3.9
LDH, IU/l					
T-PC	136 ± 28	132 ± 29*	144 ± 29	153 ± 36*	164 ± 39
C-PC	151 ± 26	150 ± 26	159 ± 25	166 ± 28	164 ± 33
Glucose, mmol/10^{12} cells					
T-PC	11.9 ± 0.8*	11.7 ± 1.0*	7.5 ± 1.7*	4.4 ± 1.0*	0.7 ± 1.1*
C-PC	15.2 ± 1.8	14.3 ± 1.8	12.6 ± 1.1	11.2 ± 1.7	9.0 ± 1.6
Lactate, mmol/10^{12} cells					
T-PC	4.5 ± 0.8	7.9 ± 1.0*	15.6 ± 3.7*	21.8 ± 1.7*	30.0 ± 2.3*
C-PC	4.3 ± 0.6	5.8 ± 0.9	10.6 ± 2.4	13.9 ± 2.0	17.5 ± 2.4

*p < 0.05; T-PC (PRT-treated unit) versus C-PC (untreated control unit).

plasma. Values indicate again a higher consumption of glucose in T-PC. With the exception of day 2, the glucose consumption rate was significantly higher for T-PC compared to C-PC. Values ranged from 0.07 ± 0.031 versus 0.040 ± 0.028 mmol/10^{12} cells/h (day 2, p = 0.063), 0.064 ± 0.033 versus 0.036 ± 0.027 mmol/10^{12} cells/h (day 4, p = 0.018), 0.067 ± 0.011 versus 0.033 ± 0.012 mmol/10^{12} cells/h (day 6, p = 0.018) to 0.069 ± 0.009 versus 0.036 ± 0.010 mmol/10^{12} cells/h (day 8, p = 0.018).

Comparable to series I, lactate results per 10^{12} cells were generally lower in C-PC (table 4). Lactate production was higher in T-PC, and the lactate production rate was significantly higher for T-PC throughout storage. Values (T-PC vs. C-PC) ranged from 0.142 ± 0.027 versus 0.063 ± 0.020 mmol/10^{12} cells/h (day 2, p = 0.018), 0.156 ± 0.054 versus 0.090 ± 0.033 mmol/10^{12} cells/h (day 4, p = 0.018), 0.146 ± 0.012 versus 0.083 ± 0.013 mmol/10^{12} cells/h (day 6, p = 0.018) to 0.150 ± 0.011 versus 0.080 ± 0.011 mmol/10^{12} cells/h (day 8, p = 0.018).

Platelet Activation

– *Series I:* We investigated the baseline expression of the platelet surface marker CD62p and the platelet maximal activation capacity using TRAP. The results of the baseline expression were significantly higher for T-PC throughout the entire storage period (table 3). On day 0 before PRT, both T-PC and C-PC showed the same maximal CD62p expression. From day 1 on, the activation capacity was significantly reduced for T-PC. In the morning of day 8, the C-PC as well as the T-PC showed again comparable expression levels (table 3). The difference between baseline and TRAP-induced CD62p expression was significantly higher in C-PC on day 5 than in T-PC.

Soluble sCD62p was significantly different only in units after RB addition and before UV illumination compared to units after plasma addition (127 ± 45 vs. 102 ± 52 ng/ml, p = 0.034). Throughout storage, values increased in T-PC and C-PC and reached levels of 135 ± 42 versus 152 ± 56 ng/ml on day 5 (p = 0.041) and 155 ± 39 versus 162 ± 62 ng/ml (p = 0.638) on day 8.

LDH release from the cytoplasma is related to shear stress. In series I, C-PC showed higher LDH levels until day 5 of storage, but not on day 8 of storage (table 3).

– *Series IV:* CD62p baseline expression levels were significantly higher in the T-PC throughout the entire storage period (table 4). CD62p expression after TRAP activation was identical in T-PC and C-PC on day 1. After PRT treatment, the activation capacity was significantly reduced in T-PC up to day 6. In the morning of day 8, the C-PC and T-PC showed again comparable levels (table 4). Comparable to series I, baseline versus maximal expression demonstrate that T-PC at baseline (61.9 ± 9.6%) and maximal activation (66.1 ± 7.2%) on day 8 is equal (fig. 3) while the C-PC still show a significant difference between baseline (26.8 ± 3.9%) and TRAP-induced CD62p expression (71.1 ± 3.9%) (fig. 4).

No differences in sCD62p levels could be found for T-PC and C-PC throughout storage. Values increased for T-PC from 105 ± 23 (day 1) to 182 ± 28 ng/ml (day 8) and for C-PC from 107 ± 31 (day 1) to 189 ± 44 ng/ml (day 8).

LDH levels were higher in C-PC until day 5 of storage, but not on day 8 of storage (table 4).

Discussion

The PRT uses RB and UV light. The aim of the study was to evaluate the practicality of this technique and its impact on in vitro platelet quality.

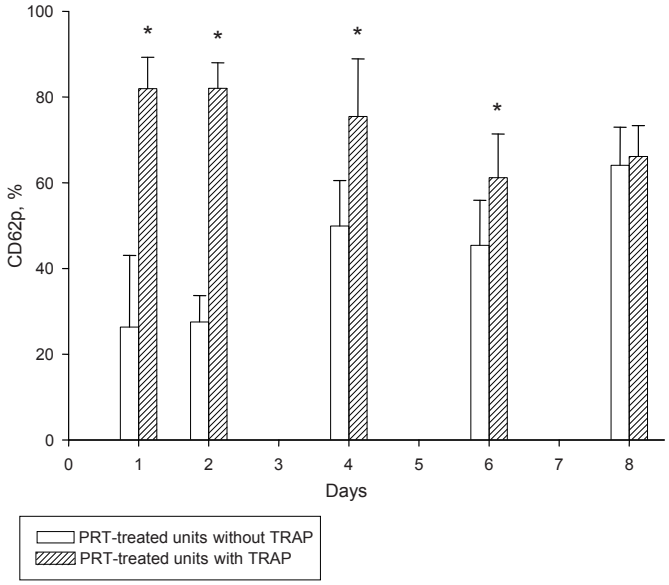

Fig. 3. Series IV (n = 6): PRT-treated units; CD62p expression (%) with and without TRAP. *p < 0.05.

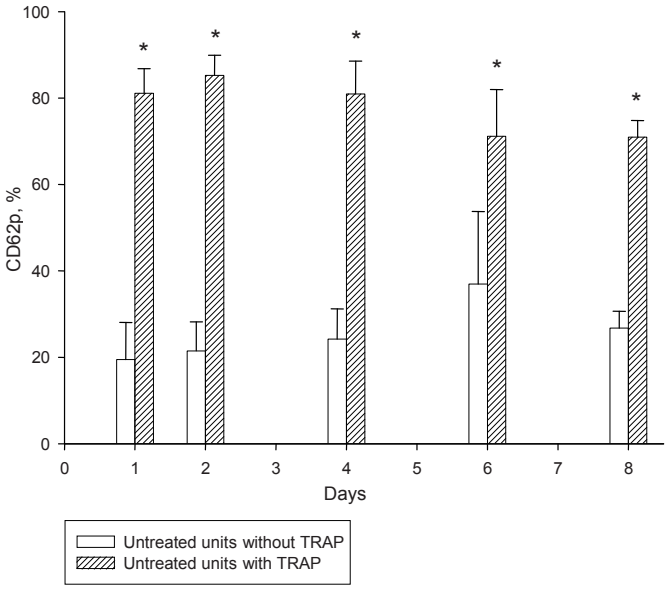

Fig. 4. Series IV (n = 6): untreated units; CD62p expression (%) with and without TRAP. *p < 0.05.

The handling procedure can be easily integrated into PC manufacturing and takes on average less than half an hour until the PC is ready for transfusion. Platelets collected by the Amicus platform have to be transferred to an illumination/storage bag (CaridianBCT). After RB addition and UV illumination, no further platelet manipulation and transfer is necessary. The platelet unit can be directly transfused.

Several publications described that platelet exposure to mechanical or chemical influences results in changes, which is collectively referred to as platelet storage lesion (PSL) [7–9]. The question arises whether PRT treatment might also lead to PSL, and if it does, to what extent.

Our results demonstrate platelet alteration due to this treatment by means of higher glucose consumption and lactate production rates indicating accelerated glycolysis. We could confirm findings from Ruane et al. [4] and AuBuchon et al. [10] who described an increased metabolic activity as a result of PRT, though in both studies the glucose consumption and lactate production rates were lower. Beyond day 5 of storage, we could not detect any remaining glucose. This is in contrast to results from Li et al. [11] who detected glucose levels of 8 ± 2 on day 4 or day 5, and 6 ± 2 mmol/l 10^{12} cells on day 6 or 7.

The accumulation of the lactic acid resulted in a decline of pH to levels of 6.7 (day 5) after PRT. This value was lower in comparison to 7.4 (day 5) observed in other studies [4, 10, 11], although the same storage bag was used and platelets were stored in plasma in all studies. Due to increase protons, our data showed reduced HCO_3^- values in PRT-treated PC up to day 5 (3.8 mmol/l), indicating limited buffer capacity.

Moreover, we could demonstrate that PRT leads to increased activation of platelets. In contrast to other studies, data from study I (n = 14) showed that the addition of RB alone activated platelets by increasing baseline CD62p expression. Values obtained from other studies (n = 18) after initial prolonged resting time (study II/II/IV) showed non-significant increases in p-selectin expression in T-PC compared to C-PC. In addition, we found higher baseline expression of CD62p throughout storage, which is comparable to published results on PRT-treated platelets [4, 10–12]. Values observed in these studies range from 35% (day 1) up to 60% (day 5), which is comparable to our values of 23% (day 1) to 35% (day 5) in series I and of 28% (day 1) to 45% (day 6) in series IV.

Furthermore, we found a decline of the activation capacity in response to agonist stimulation using TRAP. It has been described that reduced responsiveness diminishes adhesion, secretion, and aggregation in vivo [13, 14].

The correlation between the extent of platelet activation in vitro and in vivo parameters is discussed controversially [15–18]. Some authors found a positive correlation between increased activated platelets in vitro and the shortness of survival in vivo [15, 16]. Others described little evidence that the degree of platelet activation impairs the ability of transfused platelets to produce acceptable post-transfusion recovery or decreased bleeding [17, 18]. Michelson et al. [17] demonstrated in baboons that surface-expressed CD62p is rapidly cleaved from the platelet membrane and released into the plasma pool in transfused, activated, degranulated platelets. The activated platelets continue to circulate and function without affecting the life span in comparison to unactivated platelets. This could also be confirmed by Berger et al. [18] who carried out experiments with wild-type and p-selectin knockout mice. In contrast, it has been published that enhanced expression of CD62p leads to platelet recognition by macrophages of the reticuloendothelial system. This resulted in an increased formation of platelet-leucocyte aggregation through the PSGL-1

receptor and subsequent clearance from the circulation [19–21]. Some investigators have suggested that soluble sCD62p is a more sensitive marker for platelet activation than CD62p surface expression [22]. We could not detect any difference in sCD62p levels due to PRT, but we found increased baseline levels and decreased activation capacity that was more prominent after PRT treatment.

To evaluate PSL by means of platelet cell integrity loss, LDH, which can be found in the cytoplasma of platelets, was investigated. Values of treated and untreated PC were comparable and in range with published data from Klinger et al. [23]. Therefore, we conclude that no increased lysis of platelets due to PRT occurs.

The collection technique itself might have an impact on the extent and progression of PSL. We therefore extended the resting time of the PC on agitation before PCT was carried out. Nevertheless, findings on platelet metabolism and activation could not be improved. We conclude that the reason for platelet activation seen after PRT lies in the underlying mechanism of action.

In summary, PRT leads to increased platelet metabolism and activation resulting from the underlying mechanism of action and can not be improved by longer rest times of the platelets before treatment. Due to low pH values, shelf-life of PRT-treated platelets stored in plasma is limited to 5 days, which is in line with the manufacturer's recommendations and national guidelines in most European countries [24, 25]. Furthermore, it needs to be investigated whether other platelet preparation methods may improve platelet quality. A first clinical evaluation of PRT-treated platelets in thrombocytopenic patients showed that patients receiving treated platelets had comparable haemostasis and support requirements. A significant difference in corrected count increment (CCI) 1 h was observed, but the CCI 24 h, number of platelet transfusions, total platelet dose, and number of RBC transfusions per subject were not significantly different [26].

Disclosure

The authors declared no conflict of interest.

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