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Effect of mirasol pathogen reduction technology system on immunomodulatory molecules of apheresis platelets

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ABSTRACT

Pathogen inactivation for platelets by riboflavin system (MIRASOL) efficiently reduces transfusion related pathogen transmission. However little is known about its impact on platelets' immunomodulatory biochemical profile. We aimed was to assess the effects of MIRASOL treatment on platelet quality parameters and immunomodulatory molecules CD62P, RANTES, and CD40L in Single Donor Platelets (SDPs) resuspended in plasma (SDP-P) or T-PAS and additive solution (SDP-A). Twenty nine SDPs (15 SDP-P and 14 SDP-A) were included in the study. Samples were collected before, after MIRASOL treatment and just before transfusion. P-selectin (CD62P), RANTES, and CD40L were tested by ELISA. Platelet products quality assays were also performed. Platelet count/unit decreased after Mirasol treatment by 13 %. The pH of all units decreased over the 5-day storage period but remained above expected limits and the swirling test was positive throughout storage. P-selectin levels were not different between the three different time points in both SDPs-P and SDPs-A while RANTES levels were found to differ statistically significantly at the three different time points in all units and in the SPD-A subgroup. CD40L levels in all SDP products increased slightly during storage but this was not statistically significant. CD62P, RANTES, and CD40L in all time points were elevated in SDPs-A compared to SDPs-P but not at a statistically significant level. In conclusion MIRASOL treatment apart from RANTES increase does not seem to substantially affect platelets associated other cytokines and immunomodulatory molecules namely P-selectin and sCD40L which are implicated in immune transfusion reactions.

1. Introduction

Pathogen inactivation for platelets and plasma using riboflavin and UV light to introduce irreparable lesions into nucleic acids and thereby pathogens and WBC replication by riboflavin system (MIRASOL) has been proven to be effective against numerous bacteria, viruses, and parasites [1–3]. However little is known about the impact of such a processing method of blood derivates on platelets' immunomodulatory biochemical profile.

The main feature of platelet storage lesion seems to be platelet activation. Surface P-selectin (CD62P) during platelet activation is dramatically increased, and is accompanied by a simultaneous increase in the expression level of plasma soluble CD62P, which plays an important role in the initiation, formation, and expansion of the thrombus. P selectin is thus indicative of platelet activation [4].

RANTES (regulated on activation, normal T cell expressed and secreted) is an inflammatory regulator released from platelets during storage, modifying leukocyte and endothelial responses to a range of different inflammatory stimuli [5], due to their ability to attract and stimulate human eosinophils and to induce histamine release from human basophils. Its accumulation in platelet concentrates blood products has been associated with the occurrence of allergic transfusion reactions [6].

CD40L is an inflammatory regulator, that has been implicated in

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thrombosis and inflammation processes and there are indications that CD40L accumulation during storage in platelet units may be involved in TRALI [7].

The aim of the present study was to assess the effect of the riboflavin pathogen inactivation system MIRASOL (based on the riboflavin and UV light exposure) on platelet-associated cytokines and immunomodulatory molecules CD62P, RANTES, and CD40L accumulated in platelet concentrates.

2. Materials and methods

Twenty nine 29 Single Donor Platelets (SDP) were included in our study. Before apheresis all volunteer donors were tested and had normal hemoglobin levels, platelet, leukocyte count and no signs of infection, as required. Platelets were collected by TRIMA (Version 6.0, TERUMOBCT). Fifteen SDPs were re-suspended in plasma (SDP-P) and 14 SDPs in additive solution T-PAS (SDP-A). Afterwards PLT units were treated with Mirasol-PRT. Namely the MIRASOL System (Mirasol PRT System: MirasolTM, TerumoBCT, Lakewood, CO, USA) that uses light and the naturally occurring compound riboflavin (vitamin B2), to reduce the pathogen load in donor blood components and to inactivate white blood cells. It requires riboflavin to be mixed into the blood component, which is then exposed to UVA +B light (at spectra of 265–370 nm) for a short period of time (typically less than10 minutes). This illumination causes a chemical reaction that prevents DNA and RNA replication. Viruses, bacteria and other cells with nuclei (as white blood cells) can thus be inactivated [8].

CD62P, RANTES, and CD40L were tested on platelets supernatant samples in three different time points: (a) 2 h after completion of the apheresis procedure and before treatment with MIRASOL after standing at 22 °C (T0), (b) after MIRASOL treatment (T1) and (c) just before platelet release for transfusion (T2).

Platelet quality assays i.e. platelet concentration volume, platelet count, MPV, pH and swirling were also performed. Samples were aseptically drawn from the platelet concentrates. Platelet concentration and MPV were immediately evaluated using a CBC analyzer. Measurements for pH were performed at 22 °C (pH meter precision stick model PH-110). Platelet swirling was assessed visually and given a numeric score (range 0–3) based on previously described methods at the predetermined time points T0, T1 and T2. A centrifugation procedure was applied to obtain the supernatant Platelet Poor Plasma samples that were stored at − 80 °C until assayed. Measurements of sCD62P, RANTES, and CD40L were performed in supernatant samples after thawing by ELISA (R & D, Minneapolis, USA) which employs the quantitative sandwich enzyme immunoassay technique according to the manufactures instructions. Briefly, samples were added to microplates pre-coated with capture antibody and any analyte present was bound by the immobilized antibody. After washing, a detection antibody was added and was bound to the captured analyte. After washing, a substrate solution was added and a color developed in proportion to the amount of analyte present in the sample that was measured.

All patients that received platelet transfusions were monitored for adverse transfusion reactions.

The statistical analysis was performed by programming in SAS 9.4 for Windows (SAS Institute Inc. NC, USA) [9]. In order to evaluate the differences of the measured medical quantities expressed in a numeric form the Wilcoxon rank-sum test was performed in cases of paired comparisons (i.e. between the time points T0 vs T1, T0 vs T2 and T1 vs T2). When comparisons were extended in three groups (T0 vs T1 vs T2), the Friedman test was used, while when comparisons were between different groups (i.e. SDP-P vs. SPD-A) the Kruskal-Wallis test was performed. The statistical significance level was set to 0.05 and all tests were two tailed.

Table 1
Quality parameters (Mean Value ± SD) before and after MIRASOL treatment.

Variable	SDPs	T0	T1	T2	Significance of group difference over time (p-value)
Platelet Concentration ($x10^6$/ml)	N = 29	1291.15 ± 248.86	1132.31 ± 166.24	na	$p = 0.006$
pH	N = 29	7.1 ± 0.1	7.1 ± 0.1	6.8 ± 0.1	$p < 0.001$
Swirling scores	N = 29	3.0 ± 0.0	3.0 ± 0.0	2.6 ± 0.0	$p < 0.001$
Volume (ml)	N = 29	330.66 ± 29.81	366.34 ± 30.13	na	$p < 0.001$

NA: Not Available

Table 2
P-selectin, RANTES and CD40L levels in Single Donor Platelets SDP-P and SDP-A and relevant comparisons of the measured quantities over time.

Variable	SDP TYPE	T0	T1	T2	Significance of group difference over time (p-value)
P-Selectin/ CD62P ng/ml	Total SDPs	167.14 ± 53.03	177.84 ± 47.8	164.31 ± 26.20	0.5194
	SDP-P	160.89 ± 59.67	168.65 ± 43.91	161.72 ± 31.65	0.6271
	SDP-A	173.84 ± 46.15	187.69 ± 51.4	167.09 ± 19.57	0.3951
	p-value*	0.3480	0.2752	0.7270	
CCL5/ RANTES ng/ml	Total SDPs	34.12 ± 5.35	36.29 ± 6.24	33.05 ± 6.36	**0.0006**
	SDP-P	33.96 ± 3.33	35.19 ± 6.41	32.7 ± 5.72	0.0849
	SDP-A	34.3 ± 7.04	37.47 ± 6.05	33.45 ± 7.24	**0.0036**
	p-value*	0.1904	0.4850	0.8719	
CD40 Ligand ng/ml	Total SDPs	17.1 ± 7.44	17.77 ± 6.72	18.00 ± 4.70	0.2606
	SDP-P	15.95 ± 7.72	15.72 ± 4.45	16.81 ± 4.08	0.1266
	SDP-A	18.33 ± 7.20	19.97 ± 8.12	19.28 ± 5.13	0.6065
	p-value*	0.1761	0.1161	0.1378	

Mean value and standard deviation for each variable at each time point are depicted for all samples or separately SDP-P and SDP-A. P-values in bold indicate statistical significance in paired comparisons over time.

* p-value is for comparison between SDP-P and SDP-A for each parameter on each individual time point.

3. Results

Quality parameters (Mean Value ± SD) before and after MIRASOL treatment (Table 1) were as follows: Volume 330.66 ± 29.81 vs 366.34 ± 30.13 ml ($p < 0.001$), Platelet concentration 1291.15 ± 248.86 × 10^6/ml vs 1132.31 ± 166.24 × 10^6/ml ($p = 0.006$) and MPV 7.010 ± 0.71 vs 7.146 ± 0.70fl ($p = 0.4657$). The pH levels of all units (both SDP-P and SDP-A) before and after MIRASOL treatment (at T0 and T1) was 7.1 ± 0.1 (mean ± SD) and 6.8 ± 0.1 just before transfusion. The pH of all units decreased over the 5-day storage period but remained within the limits defined by the Council of Europe directives (pH> 6.4) up to the release of the product. All units had a positive swirling test throughout storage. More specifically before and after Mirasol treatment the mean swirling score for both before and after Mirasol treatment was 3.0 ± 0.0 while Swirling scores on the day of transfusion were somewhat

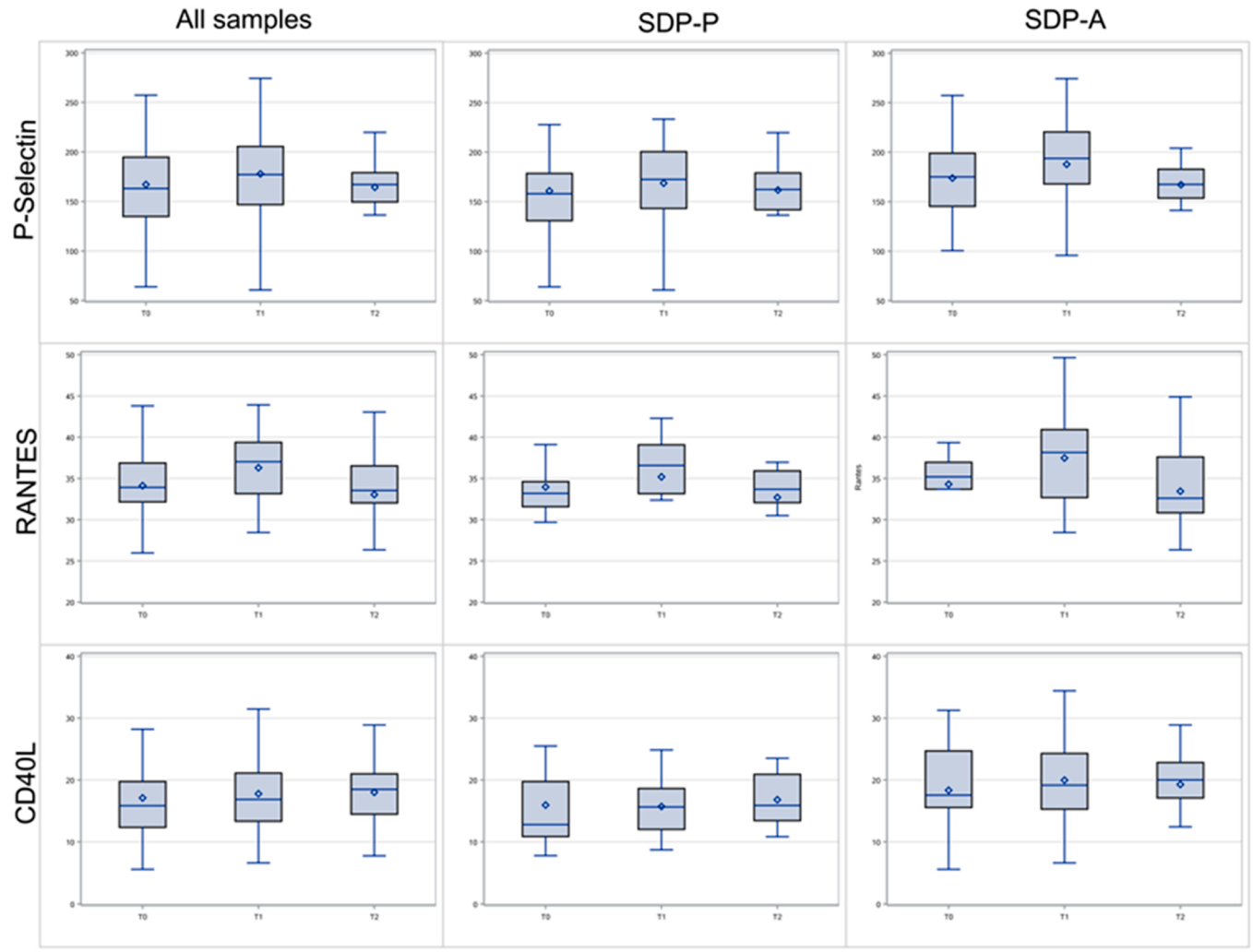

Fig. 1. Box and Whisker plots for the levels of P-Selectin, RANTES, CD40L. Top row: P-Selectin, middle row: RANTES and bottom row: CD40L. From left to right: All samples, SDP-P and SDP-A samples. For each box the low and high limit indicate the 25th and 75th percentile, the lines within the boxes the median value and the diamond symbol the mean value. Whisker limits indicate the minimum and maximum values after excluding outliers.

lower (mean score 2.6 ± 0.0). The mean time of storage of platelets products in our study was 4.5 ± 1.1 days.

CD62P, RANTES, and CD40L were evaluated in a total of 29 SDP (15SDP-P and 14 SDP-A samples) in the three different time points T0, T1, T2. The descriptive statistics (mean values, standard deviation end for completeness reasons additional statistical quantities) are presented in Table 2. Relevant Box and whisker plots for all samples and separately for SDP-P and SDP-A at the three time points are depicted in Fig. 1. It is worth noticing that no adverse transfusion reactions related to the transfusion of platelets products in our study were recorded.

Regarding P selectin levels no significant difference was found between the three different time points (T0, T1, T2) in the total number of samples as well as in both subgroups SDP-P and SDP-A. RANTES levels in the total number of samples and in the SPD-A subgroup at the three different time points were found to differ statistically significantly ($p = 0.0006$ and $p = 0.0036$ respectively). However this difference was not possible to be confirmed in the SDP-P subgroup ($p = 0.0849$).

CD40L levels in all SDP products increased slightly during storage, however, there was no significant difference of CD40L in the three time points either when examining all the samples together or for the individual SPD-P and SDP-A subgroups.

Furthermore, in order to evaluate whether the SDP type has a role in the levels of measured variables, we compared for each individual time point the levels of those variables between SDP-P and SDP-A. No statistically significant difference was found in the levels of P selectin, RANTES and CD40L between SDP-P and SDP-A for any time point, the relevant p-values are depicted in Table 1.

4. Discussion

Contamination of blood products with viruses, parasites, bacteria or emerging pathogens can compromise blood safety. Pathogen reduction systems can reduce the risk of contamination of blood products. PRT treated platelets seem to be efficient in vivo and many in vitro quality tests (such as pH, lactate production, glucose consumption rate, pO2, pCO2, p-selectin expression, swirling) have been proposed so far in order to predict in vivo platelet performance. In our study we evaluated pH, swirling along with p selectin, RANTES and CD40L as quality markers of Mirasol treated platelets.

Even though there was a reduction of 13 % of the platelet concentration as previously described [3,10], the total platelet content per unit (yield) after MIRASOL treatment was $3.83 \times 10^{11} \pm 5.4 \times 10^{10}$ which is equivalent to a therapeutic platelet transfusion dose. According to Middelburg et al. [10] PLT concentration declined by almost one-fifth in Mirasol-treated PLTs, while the dilution factor from addition of the riboflavin solution should be less than 1/10, however survival of the remaining PLTs was not affected.

Knowing that p-selectin is stored in the PLT α granules with other

cytokines and growth factors, the levels of soluble p-selectin can be expected to mirror the levels of cytokines released [11].

It has been reported by Castrillo et al. [12] that Mirasol-treated platelets (Buffy Coat platelet concentrates in PAS) are more activated than untreated controls, as evidenced by elevated levels of CD62p expression on the cell surface at all storage time points. In the same study soluble CD62p levels were also elevated especially at the end of storage [12]. Soluble CD62p levels in our study in both plasma and PAS platelets were also elevated at T1 (after Mirasol treatment) as previously described [13], but not at a statistically significant level and no increase was noted during storage. A significant increase of levels of p-selectin has been recorded especially on day 7 of storage [11] and the shorter storage time of PLTs in our study could account for our findings.

Regarding in vitro quality measures after 5 days of storage it has been shown that Mirasol PRT treatment minimally influenced in vitro properties of apheresis platelets, as in our study. On Day 6, however, surface P-selectin was expressed more in Mirasol Treated-PCs and swirling was poor, but the mean pH remained above 6.4. As compared with 5-day-stored platelet, a significant decrease in mean pH values and swirling scores was observed on Day 7 as well as a significant increase in P-selectin expression. However, the observation that Mirasol PRT-treated platelets continue to consume glucose throughout the storage indicates that platelets remain functional despite low pH [12–14].

RANTES levels increased after MIRASOL treatment in our study and were within the range reported by others. During storage time RANTES levels have been reported to be both increased and unchanged[11, 15, 16] as in our study. In patients following platelet transfusions, RANTES has been associated with non hemolytic and allergic transfusion reactions [6, 11, 17]. The reported RANTES levels associated with allergic reactions range from approximately 200 to 1000 ng/ml with a median of 650 ng/ml [6]. According to Picker et al., 2009 [11] RANTES levels detected in Mirasol-PRT-treated units were elevated (median of 360 ng/ml for 5-days-stored PLTs) compared to untreated control units (median of 195 ng/ml), but within a range where the risk of inducing an allergic transfusion reaction may be low. Although RANTES increased after Mirasol treatment its levels return to pre-treatment level at the time of transfusion a finding that renders the contribution of PRT to the development of allergic reactions unlikely compared to standard platelet products. That was confirmed in our study by the fact that no allergic reaction was recorded after transfusion [12,18].

sCD40L, which is cleaved from the surface of activated platelets [19, 20], increases over the PC storage period [7,21]. MIRASOL treatment was also found to increase the accumulation of sCD40L in platelets concentrates [15, 22, 23]. Although our results show a trend of increasing total sCD40L levels over time, this was not statistically significant. Higher levels of sCD40L than our study results in platelet products have been associated with adverse transfusion reactions such as febrile, allergic reactions, and TRALI, suggesting that it is possible that the transfused units containing more elevated levels of sCD40L contribute to the development of TRALI [7].

Concerning the storage medium used in platelet concentrates (plasma or additive solution) that could influence several parameters, its role should be taken into consideration especially when cell lysis or activation parameters are discussed. All immunomodulatory parameters assessed in our study in all time points were elevated in platelet units stored in AS, SDP-A compared to platelets stored in plasma SDP-P but not at a statistically significant level. Accordingly studies comparing PAS to 100% plasma, showed higher levels of CCL5 (RANTES), CXCL4 (PLT factor 4), TGF-β1, and PLT activation markers when PLTs were stored in PAS rather than 100% plasma, indicating the protecting role of plasma in platelet lysis and activation during storage [11,24].

The absence of a control group concerns a limitation of our study, however PLTs before Mirasol treatment served as an internal control of Mirasol effect.

5. Conclusions

In conclusion our data analysis suggests that MIRASOL treatment apart from RANTES increase does not seem to substantially affect PLTs associated other cytokines and immunomodulatory molecules namely P selectin and sCD40L, which are implicated in immune transfusion reactions. Further clinical studies are needed to elucidate the role of platelet-derived immunomodulatory mediators and plasma reduction regarding the occurrence of adverse transfusion reactions.

CRediT authorship contribution statement

M.P and EG designed the study, DM, MPG,TP,AR performed the apheresis, collected and analysed the samples, provided the demographic data, AP performed the statistical analysis, SV, EG, and MP analysed the data, SV and MP wrote the paper.,All authors critically reviewed the paper and accepted it in its current form.

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