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Comparison between in vitro properties of washed platelet concentrates suspended in M-sol and those in BRS-A, both of which were prepared with an automated cell processor

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ABSTRACT

Background: Washed platelet concentrate (WPC) is prepared manually in general, but automated preparation is desirable to minimize variation in the WPC quality and enhance WPC production. Recently, the software was improved for an automated cell processor (ACP) to control all processes of WPC preparation. M-sol and BRS-A, which are mixtures of medical solutions, are widely used for WPC preparation with a manual method in Japan. In this study, we prepared WPC suspended in M-sol (WPC-M) or BRS-A (WPC-B) with the ACP, and compared their in vitro properties during 7-day storage.
Study design and methods: PC was divided into two equal aliquots for WPC-M and WPC-B. A divided PC, medical solutions and disposable materials were set in the ACP, and it was started to prepare WPC-M or WPC-B on Day 0. Prepared WPC was stored on a flatbed shaker until Day 7.
Results: The pH of WPC-M and WPC-B was maintained above 6.8 during the 7-day storage. The differences in aggregation (%), HSR (%), P-selectin expression, GPIbα expression, and phosphatidylserine expression between WPC-M and WPC-B were minimal until Day 3.
Conclusion: The in vitro properties of WPC-B are not markedly different from those of WPC-M until Day 3.

1. Introduction

Washed platelet concentrate (WPC) is useful for preventing transfusion-related adverse reactions caused by the plasma component in conventional platelet concentrate (PC) [1–6]. WPC is usually prepared by removing almost all plasma from centrifuged PC and adding additive solution to the pellet (manual method). The manual method requires some skill; therefore, plasma removal (%) on WPC preparation depends on the skill of the worker. To minimize variation in the WPC quality, the PC washing process should be automated, which may also lead to the enhancement of WPC production. Buck et al. previously reported that 94% of plasma in PC was removed with a blood cell processor, and the prepared WPC suppressed febrile and allergic reactions [1].

The automated cell processor (ACP) is already being used to wash red blood cells at all product departments of blood centers in Japan. Utilizing the ACP for washing not only RBC but also PC will make it unnecessary to purchase a new medical instrument for WPC preparation. It was reported that WPC suspended in M-sol additive solution (WPC-M) [7–10], which is a mixture of medical solutions, could be prepared with the ACP [11] although the ACP itself had no function to mix medical solutions. To increase the convenience, software of the ACP was recently improved so that mixing of medical solutions could be performed automatically.

There are no commercially available additive solutions for WPC in Japan. M-sol, which is prepared by mixing five commercially available medical solutions, has been widely used for WPC preparation with a manual method in Japan since 2005 [3,4,6]. M-sol is not on the market; therefore, it has to be prepared in advance even if the improved ACP is used for WPC preparation, because the improved ACP is structurally unable to mix more than three medical solutions.

Abbreviations: ACP, automated cell processor; HSR, hypotonic shock response; MPV, mean platelet volume; PC, platelet concentrate; PLT, platelet; WPC, washed platelet concentrate; WPC-B, WPC suspended in BRS-A; WPC-M, WPC suspended in M-sol.

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Table 1
Composition of M-sol and BRS-A (mM).

	M-sol	BRS-A
Sodium chloride	77	95.2
Potassium chloride	3.0	3.8
Magnesium chloride/sulfate	1.6	0.9
Sodium bicarbonate	44	26.6
Glucose	15	5.8
Sodium acetate	21	–
Trisodium citrate	9.4	4.2
Citric acid	4.8	1.8
Calcium chloride	1.0	1.4

BRS-A additive solution was reported to be available as an additive solution for WPC in 2013 [12]. Not only M-sol but also BRS-A has recently been widely used as an additive solution for manually prepared WPC in Japan. BRS-A is also not on the market, but it can be prepared with the improved ACP, because it involves mixing two medical solutions [12]. Therefore, BRS-A is a preferable additive solution for WPC prepared with the improved ACP, but it is unclear whether or not WPC suspended in BRS-A (WPC-B) can be an alternative to WPC-M. In this study, we compared the in vitro properties between WPC-M and WPC-B during 7-day storage.

2. Materials and methods

2.1. WPC preparation and storage

The apheresis apparatus for preparing leukoreduced PCs (plasma content: 100%) was Trima Accel (Terumo BCT, Tokyo, Japan), CCS (Haemonetics Corporation, MA, USA), or TERUSYS (Terumo BCT, Tokyo, Japan). Two ABO-identical apheresis-derived PCs from healthy donor were mixed and divided into two equal aliquots (PC for preparing WPC-M and WPC-B). M-sol was prepared as described in a previous report [7]. BRS-A was prepared by mixing BICANATE (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) and ACD-A (Kawasumi Laboratories, Inc., Tokyo, Japan) at 20:1 in ACP (ACP215, Haemonetics Corporation, MA, USA) with the WPC preparation program (215J-B.5, Haemonetics Corporation, MA, USA). The final compositions of M-sol and BRS-A are shown in Table 1. PC, ACD-A, the medical solution (M-sol or BICANATE), and platelet wash disposable set (238J-00, Haemonetics Corporation, MA, USA) were set up in predetermined positions of the ACP. The operating of the ACP was performed according to the manufacturer's protocol. Before PLT washing, ACD-A (10% of PC weight) was automatically added to PC to inhibit PLT aggregation. Prepared WPC was stored at 20–24 °C on a flatbed shaker (60 cycles/min) for 7 days.

2.2. Measurement of basic variables

The automatic blood gas analyzer for pH measurement was Rapidpoint 405 (Siemens Healthcare Diagnostics, Munich, Germany). The automated blood cell counter for measurement of the PLT count and mean PLT volume (MPV) was XS-800i (Sysmex Corp., Kobe, Japan).

PLT recovery and plasma carryover were calculated as:

$$\text{PLT recovery}(\%) = 100 \times \frac{(\text{total PLT count in WPC on Day 0})}{(\text{total PLT count in PC})}$$

$$\text{Plasma removal}(\%) = 100 \times \left[1 - \frac{\text{total protein weight in WPC supernatant on Day 0}}{\text{total protein weight in PC supernatant}}\right]$$

The supernatant protein concentrations were determined using BCA methods with the protein assay kit (BCA Protein Assay Reagent Kit, Pierce, Rockford, IL, USA).

PC and both WPCs were sampled and diluted at 300×10^9/L with autologous or AB plasma in each storage period to prepare platelet-rich plasma (PRP) for measuring aggregation, and the hypotonic shock response (HSR). The platelet aggregation test was performed using a PLT aggregometer (MCM HEMA TRACER 313 M, MC Medical Inc., Tokyo, Japan), and aggregation agents at the following final concentrations: 2.5 μg/mL for collagen (horse tendon derivation), and 5 μmol/L for ADP. Horse tendon collagen (collagen reagent Horm) was purchased from Nycomed Pharma GmbH (Munich, Germany). The HSR test was performed according to standard methods using the spectrophotometer UV-2550 (Shimadzu Corp., Kyoto, Japan) [13]. Glucose and lactate concentrations in the supernatants were measured with commercial kits for glucose (glucose CII test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and lactate (Determiner LA, Kyowa Medex Co., Ltd., Tokyo, Japan), respectively.

2.3. Measurement of PLT surface markers

The p-selectin (CD62P), GPIbα (CD42b), and phosphatidylserine (PS) expressions were measured by a flow cytometer (Cytomics FC500, Beckman Coulter, Tokyo, Japan). To measure p-selectin expression, paraformaldehyde-fixed PLTs were stained with phycoerythrin (PE)-conjugated anti-CD62P (BD Bioscience, San Jose, CA, USA) and fluorescein isothiocyanate (FITC)-conjugated anti-CD61 (BD Bioscience). The corresponding isotype antibody was used as a negative control. The CD61+ cells were counted for up to 10,000 events using a flow cytometer. The p-selectin expression was assessed as the percentage of specific CD61+/CD62P+ cells in all CD61+ cells. To measure GPIbα expression, paraformaldehyde-fixed PLTs were stained with PE-conjugated anti-CD42b (Beckman Coulter, Inc.) and FITC-conjugated anti-CD41 (Beckman Coulter, Inc.). The corresponding isotype antibody was used as a negative control. After measurement with a flow cytometer, the mean fluorescence intensity (MFI) of GPIbα was assessed to determine if changes occur during storage. To measure PS expression, PLTs were stained with fluorescence-conjugated annexin-V (annexin-V-FITC; Sigma–Aldrich, St. Louis, MO, USA), according to the manufacturer's protocol, and measured by flow cytometry to calculate the percentage of annexin V binding to PS on apoptotic PLTs.

2.4. Statistical analysis

Student's *t*-tests were performed for statistical analysis between the data of WPC-M and WPC-B. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Basic variables

The characteristics of PC and both prepared WPCs are described in Table 2. There were not significant differences in the PLT volume, PLT concentration, PLT count, PLT recovery, or plasma removal between WPC-B and WPC-M (Table 2).

pH values of both WPCs were maintained above 6.8 during the 7-day storage (Fig. 1A). pH values of WPC-M were higher than those of WPC-B during the 7-day storage (Fig. 1A).

The changes in MPV values of WPC-M from Days 1 to 7 were small, although the values of WPC-M temporarily increased immediately after washing (Fig. 1B). The difference in MPV values between WPC-B and WPC-M were negligible from Days 1 to 3, but