Original Article

Evaluation of Biochemical Parameters of Platelet Concentrates Stored in Plasma or in A Platelet Additive Solution (Composol)

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Abstract

Objective

Removing plasma from the platelet concentrate (PC) medium could be an effective way to increase the safety of this product. The goal of this study was to compare PC stored in plasma or in an additive solution (Composol) with in vitro testing.

Materials and Methods

Fifty-four single donor PCs were prepared from Iranian Blood Transfusion Organization (IBTO). Each PC unit was divided into two portions. Then in one of the portions, plasma was replaced with Composol. Sampling was carried out at the days 2, 4 and 7 from the preparation time. The levels of pH, glucose, lactate and lactate dehydrogenase (LDH) were analyzed by colorimetric methods.

Results

The levels of pH and glucose were decreased during storage whereas the levels of LDH and Lactate were increased with time over. At the day 7 of storage, the mean values for glucose were 404.44 and 25.19 mg/dl in plasma and Composol, respectively. These values were 3306.1 and 683.33 U/L for LDH and 142.07 and 90.90 mg/dl for lactate. The differences between LDH, lactate, and glucose levels were significant between the two storage media of plasma and Composol (P-value<0.001).

Conclusion

This study could imply the potential capacity of an additive solution as a candidate for plasma replacement in PC in vitro.

Key Words

Platelets, Additive solution, Lactate Dehydrogenize, Glucose, Lactates

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Introduction

The most widely used preparation of platelet concentrate (PC) is a concentrate stored in plasma and has the shelf life of 3 to 5 days if stored at 22 °C. The development of platelet additive solutions (PAS) as substitutes for plasma was initiated in the 1980s and further improvements followed in later years (1-3). PAS has been introduced starting with simplistic saline-based formulations such as T-Sol/PAS-II (Baxter Healthcare Corporation, Deerfield, IL, USA) and Plasma-Lyte (Baxter Healthcare Corporation) (4). The replacement of plasma with PAS as in PC has a number of advantages. PAS can be produced sterile, and have a standardized composition, distinct with plasma. Furthermore, the lower content of proteins reduces the allergic reactions. Also, due to absence of antibodies, ABO-incompatible transfusions can be tolerated easier. Additionally, by not using plasma for the preparation of PC, more plasma becomes available for fractionation. Finally, these additive solutions allow pathogen reduction technologies (5).

Most PC is still made with plasma, although more countries consider the use of additive solutions (6). In the evaluation of biochemical parameters in PC stored in glucose solution it was revealed that when the glucose was added at the beginning of storage (time 0), there was a recovery of ATP, GTP and a decrease of energetic catabolism, demonstrating a beneficial effect on energy metabolism. Whereas the addition of glucose 0.5% on day 5 did not produce significant differences in metabolites of energy pathways with respect to control PC (7). Both in CPD plasma and in PAS, the presence of high levels of glucose should be avoided because oxidized glucose will generate lactic acid, causing rapid decline of pH in the stored PC and most PAS therefore use acetate as platelets nutrient (8).

The latest-generation solutions are currently licensed in Europe; these include PAS-IIIM (MacroPharma) and Composol (Fresenius). Van der Meer and coworkers (9) compared the in vitro storage characteristics of pooled buffy coat platelets stored for up to 12 days in 100% plasma, or in mixtures of plasma with PAS-II, PAS-III, PAS-IIIM and Composol. They observed that several in vitro markers of platelets quality (pH $\geq$6.8, glucose consumption, lactate production) were reasonably well preserved for 9–12 days in platelets stored either in 100% plasma, or in PAS-IIIM (30% plasma) or Composol (35% plasma) (10,11).

Nonetheless, whether plateles storage in these additive solutions has beneficial effect or not still remains uncertain and under discussion. This study was carried out to to survey more on in vitro markers of platelets quality in the both media of plasma and Composol.

Materials and Methods

Sample Preparation

In this experimental study, fifty-four single donor platelet concentrate bags (JMS Singapore Pte Ltd. contained CPDA-1 solution) were prepared from IBTO (24 hours after PC preparation and completion of viral safety tests). Informed consent was obtained from the blood candidates by Iranian Blood Transfusion Organization (IBTO). Platelet rich plasma (PRP) was used to prepare PC. The count of the cells were estimated in the range of 5.1-7.2×10^{10} /bag.

Composol Replacement

The protocol for the division of PC into two portions and replacement with Composol was as below:

Each PC bag (A) was connected to two transfer bags (B and C) using a connecting device instrument (TSCD-II Terumo Sterile Tubing Welder). The transfer bags contained no anticoagulant solution. Half of the bag A constituent was transferred to the transfer bag B via transfer tube using a digital balance (Sartorius). The bag A was then separated from the B and C transfer bags by a tube sealer. After centrifugation of the A (as a control) and B bags, the

plasma of the B was transferred into the C using an extractor. Then B and C bags were separated by a tube sealer. Finally, Composol solution (Fresenius Kabi, Tabel 1) was added into the platelet pellets of B bag in the same weight of the removed plasma using a connecting device. Approximately, 20% of plasma was left in the bag. In continuation, the suspended platelets of A and B were incubated and simultaneously agitated in a shaker-incubator that was adjusted at 22°C. Sampling of the cords/bags was carried out at the days 2, 4 and 7 from the preparation time.

Biochemical Analysis

pH values were determined by the pH meter (Mettler Toledo, Switzerland). The biochemical parameters of glucose, LDH and lactate (Randox, UK) were determined using colorimetric methods by Roche Hitachi 902 Chemistry Analyzer (Roche, Germany). Calibration was carried out using calibrators of Boehringer Mannheim (Germany).

Consequently, visual observations were taken for the evaluation of small and large platelet aggregates formed in plasma or Composol after 7 days storage.

Statistical Analysis

Paired samples t- tests with SPSS 16.0 software [SPSS, Inc., Chicago IL, USA] was carried out to compare the results of this experiment. A level of $P< 0.05$ was considered statistically significant.

Results

Ph Changes during Storage of PC

The results showed a pH decline in the PC medium during storage in the Composol solution. It is worth mention that at the beginning of the experience and before the performance of the medium exchange, the primary pH value of Composol was determined 7.0. Mean and SD values could be compared in table 2.

Changes in Glucose, Lactate and LDH during Storage of PC

As it can be deduced from Table 1, the Composol solution lacked glucose. Nevertheless, the PC stored in this medium had a little level of glucose because approximately 20% of plasma remained in PC during replacement process. Glucose levels were decreased during 7 days storage in PC containing Composol or plasma (Table 2). The differences between the results of glucose in the days 4 and 7 between plasma and Composol media of PC were statistically significant (P-value<0.0001).

Lactate levels were measured in PC media at various times and showed increase during 7 days storage of PC in Composol or plasma. The data showed that the level of lactate was significantly lower in the Composol medium than that of plasma in the days 4 and 7 of storage (P-value<0.0001).

Besides, the results of this study showed that the LDH levels were also increased in PC stored in plasma or Composol with time (Table 2). The data demonstrated that the level of LDH was significantly lower in the Composol medium of PC than that of plasma in the days 4 and 7 of storage (P-value<0.0001).

Table 1. Composition of synthetic platelet additive solution [Composol]

Composition of Composol	Amount
Sodium	173 mM
Potassium	5 mM
Magnesium	1.5 mM
Chloride	98 mM
Citrate	10.9 mM
Acetate	27 mM
Gluconate	23 mM
pH	7.0-7.4) 7.2(

Table 2. Platelet parameters during time in storage

n=54			pH	LDH (U/L)	Lactate (mg/dl)	Glucose (mg/dl)
2th day	plasma	Mean	7.53	624.81	46.53	500.74
		Standard deviation	0.11733	427.594	27.8107	119.872
4th day	plasma	Mean	7.50	1567.5	102.32	442.22
		Standard deviation	0.18682	860.37	51.9484	95.615
	Composol	Mean	7.01	232.15	23.80	32.96
		Standard deviation	0.29113	180.52	16.6315	17.871
		P. Value*	p<0.001	p<0.001	p<0.001	p<0.001
7th day	plasma	Mean	7.40	3306.1	142.07	404.44
		Standard deviation	0.21263	2274.89	51.5497	103.571
	Composol	Mean	6.41	683.33	90.90	25.19
		Standard deviation	0.29088	845.56	72.6275	25.893
		P. Value†	p<0.001	p<0.001	p<0.001	p<0.001

Study on the Platelets Aggregation

At the day 7 of storage, we observed a number of small and large platelet aggregates in PC stored in plasma. On the contrary, no observable aggregates were detected in the platelets stored in Composol (Figure 1).

Discussion

Platelet additive solutions have been developed to recover plasma for other purposes, to avoid transfusion of large volumes of plasma to patients, to improve storage conditions and to allow pathogen reduction technologies, both because plasma may inhibit the effectiveness of the pathogen reduction treatment, and to maintain platelets quality after treatment (12).

According to the American Association of Blood Banks (AABB) (13), the pH level of ≥6.2 is an essential requirement for quality control of blood components. This study showed that the buffering capacity of Composol was lower than that of plasma so the pH dropped rapidly in the additive solution. On the contrary, the pH was approximately stable in plasma. The results agreed with previously reported studies for example Gulliksson H and coworkers described a rapid fall in pH in additive solution-containing media, due to the very limited buffering capacity of these media compared with that of plasma (12). In conflict, others like Sweeney J and coworkers reported a pH decline in plasma pools with storage, but either increased or constant pH in the additive solution pools (14) and Wagner SJ in 2008 reported less of a pH decrement following interrupting agitation of platelets suspended in M-sol than that of plasma (15).

Beside, the results of our study showed fall of glucose during the storage time in the both media of plasma and Composol. Fall in glucose level showed its consumption and could be an indicator for the energy generation in the cells. Presence of glucose in the Composol medium of PC could represent the remained plasma after the replacement process. Although the level of glucose was being decreased during the storage in the both media, plasma had further reduction in the glucose level. The results of this study correlated with all the related studies. For example, Sandgren P and coworkers reported significantly lower glucose concentration for platelets stored in the additive solution of InterSol (16).

Study on the lactate revealed that lactate was increased in the both media. Increasing of lactate represented the consumption of glucose for the generation of energy in the cells. Although the level of lactate was increased during the storage in the both media, there was further increscent of lactate in plasma. The results of this study were correlated with all the related researches. For example, Wagner SJ in 2008 revealed lower production of lactate during 7 days storage of PC in M-sol than that of plasma (15).

The enzyme LDH as an intracellular enzyme is often used as a marker of tissue breakdown. In this study, it was another parameter that was analyzed to show the extent of PC destruction during storage in plasma or Composol. Although LDH level was increased during the storage in the both media, the rise of LDH was significantly higher in plasma than that of Composol. Whereas Koerner K, reported an equivalent level of LDH in the media containing acetate compared to plasma during 5 days storage of PC (17). The differences in the results of various researches inevitably originated from different constituents of the utilized additive solutions.

Altogether, of this study could imply the beneficial effects of Composol compared to plasma and it seemed that cell survival could be maintained in additive solutions like Composol. Nonetheless it is worth mention that the real capacity of this additive solution as a substitute for plasma in PC only will be uncovered in living body usage.

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Conflict of Interest
None of the authors have any conflicts of interest to declare.

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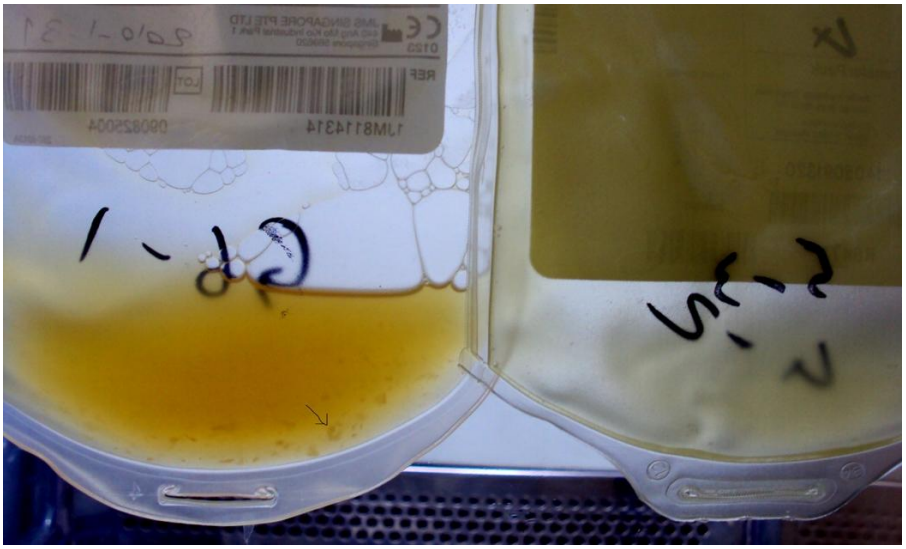

Figure 1. Visual detection of aggregates in PC suspended in plasma or Composol after 7 days of storage. Small and big aggregates can be distinguished in PC stored in plasma (arrow), whereas no detectable aggregates were observed in PC stored in Composol.