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Original Article

Effect of Platelet Additive Solution (PAS) on Random Donor Platelet Concentrates

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Abstract

Background: To observe in vitro parameters of stored platelets in PAS as compared to plasma for 10 days.

Methods: A comparative study was carried out on 42 samples of random donor platelet concentrates (PC). They were divided into two groups of 21 samples each and stored in incubator at 22°C. In one group plasma was replaced with PAS. Sampling was done on day 0, 3, 5, 7, 8, 9 and 10. Bacterial cultures were carried out. All the samples,which showed contamination, were excluded from the study.

Results: pH and glucose levels were reduced in both groups but they dropped more rapidly in PC stored in plasma than in PAS (p-value ≤ 0.001). However, the difference in LDH levels between two groups was not statistically significant. The mean values of PO_2 were reduced whereas mean values of PCO_2 were increased in both groups, but these changes were seen much less in PC stored in PAS (p-value ≤ 0.001). The results showed that difference in all parameters between two groups was highly significant (p-value ≤ 0.001) except LDH.

Conclusion: All the in vitro parameters were well preserved in PC stored in PAS than in plasma.

Key Words: Platelet additive solution PAS, PO_2, PCO_2.

Introduction

Different types of platelet additive solutions (PAS) are used to store platelets. Platelets are usually stored in donor plasma at 22- 24°C. However, even under ideal conditions, the maximum life span of platelet concentrates is 3- 5 days in vitro.[1,2] A lot of research has been carried out to replace donor plasma with a medium which can increase platelets survival and functional capability. Such solutions are called platelet additive solutions (PAS).[3] The PAS includes components with specific effects on platelets. Such components are not present in plasma or anticoagulant.[4] The development of PASs started in 1980s and are being continuously improved.[5] A number of PAS (PAS- G, T- sol, viacyte, PAS- SSP, Plasmalyte- A, PAS- I, PAS- II etc.) have undergone experimental evaluations.[6] PASs can be prepared by addition of different compositions of Na, K, Mg, acetate, citrate, gluconate and phosphate.[7] The use of PAS has several advantages. It reduces transfusion of unwanted antibodies and increases more plasma available for fractionation.[8] In different studies, it has been seen that different *in vitro* parameters of stored platelets like pH, platelet count, PO_2 and PCO_2 are much better in PAS than in donor plasma .[9, 10] In these studies, the use of PAS enhanced the shelf life of stored platelets to 9- 14 days.[11]

Materials and Methods

Forty two platelet concentrate samples of healthy individuals fulfilling the donor criteria were prepared, which were divided into two groups, one with PAS and other without PAS. Standard protocols were followed to assess the in vitro parameters of platelets. Whole blood was taken into 450 mL triplet JMS bags containing CPD A1 anticoagulant and random donor platelet concentrates were prepared within six hours of collection. Platelet rich plasma was separated from whole blood by light spin centrifugation at 2500 rpm for 3 minutes at 20°C, 50- 70 mL of platelet concentrates were obtained from single blood bag. In 21 samples, approximately 35- 50 mL of plasma was removed and equal volume of PAS was added and other 21 samples were left with plasma. These samples were kept in platelet incubator at 22°C and 3mL sample was taken from both groups on day 0, 3, 5, 7, 8, 9 and 10 (Table 1).The glucose and LDH levels were determined by Micro lab 300 at 546 nm and 340 nm, respectively. The biochemical analysis of pH, PO_2 and PCO_2 was performed, using Gas analyzer (Rapid Lab Analyzer 348). Bacterial cultures were carried out on all the platelet concentrates on day 0, 5 and 10.Independent t-test was carried out to evaluate the in vitro parameters of platelet concentrates with and without PAS on day 0, 3, 5, 7, 8, 9 and 10. Analysis of

variance (ANOVA) for repeated measures was also used to analyze the data. A *p*-value of ≤ 0.05 was taken as statistically significant.

Table I: Composition of different PAS (mmol/l)

Chemicals	Plasmalyte	PAS II	PAS III	PAS IIIM	Composol
Nacl	90	115.5	77.3	69.3	90
KCl	5	-	-	5	5
MgCl	3	-	-	1.5	1.5
NaCitrate	-	10	10.8	10.8	27
Na acetate	27	30	32.5	32.5	27
Na phosphate	-	-	28.2	28.2	-
Na Gluconate	23	-	-	-	23

Results

The pH decreased in both groups during storage but this reduction was more in platelet concentrates stored in plasma (*p*-value ≤ 0.001) (Figure 1) .The mean values of glucose in platelet concentrates stored without PAS were notably decreased during 10 days storage. The difference between two groups became statistically significant on day- 5 (Table- 2).

Table- 2: Glucose level of platelet concentrates with and without PAS (mmol/ L)

Platelet Storage Days	Glucose levels		p value
	Without PAS	With PAS	
Day 0(Baseline)	14.8± 0.7	15.1± 0.8	0.23
Day 3	13.9 ± 0.7	14.6± 0.7	0.01
Day 5	13.1± .8	14.0± 0.6	0.01
Day 7	11.5± 0.7	13.4±0.7	0.001
Day 8	10.3± 0.5	12.4± 0.9	0.001
Day 9	9.4± 0.5	11.3± 1.1	0.001
Day 10	8.3±0.3	10.2± 0.9	0.001

Results shown as mean ± SD of 16 samples without PAS and 16 samples with PAS by using independent-*t*- test.

Table-3: LDH level of platelet concentrates with and without PAS (µ/ L)

Platelet Storage Days	LDH level		p value
	Without PAS	With PAS	
Day 0 (Baseline)	140.6± 6.9	144.0±6.6	0.16
Day 3	144.5 ± 6.2	148.1 ±6.5	0.11
Day 5	148.7± 6.6	152.5±.6.0	0.1
Day 7	154.1±8.4	157.1±5.8	0.25
Day 8	160.5±9.0	162.5±6.4	0.49
Day 9	173,7.1±9.3	175.3± 7.3	0.58
Day 10	172.6± 10.1	171.9± 8.1	0.82

Results shown as mean ± S.D of 16 samples without PAS and 16 samples with PAS by using independent- *t*-test.

DH levels increased in both groups during 10 days storage but the difference was insignificant (Table- 3).

The PO_2 levels increased in both media but this increase was seen more in plasma and the difference between two groups was statistically significant (Figure-2). PCO_2 levels were measured at various times and showed a significant decline in PCO2 levels in PC without PAS(Figure-3).

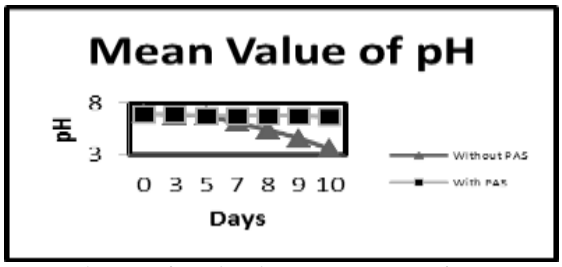

Figure-1: The pH in platelet concentrates in two groups (with and without PAS)s. The difference between two groups during 10 days of repeated measurements using ANOVA started becoming significant by day 7.

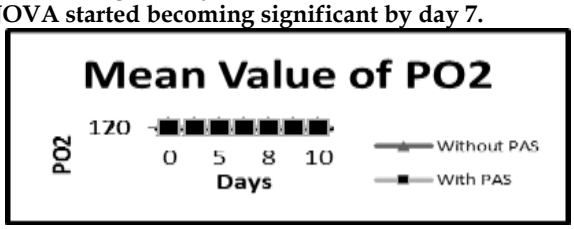

Figure-2: PO_2 in random donor platelet concentrates in two groups (with and without PAS)s. The difference between two groups of repeated measurements using ANOVA started becoming significant by day 7.

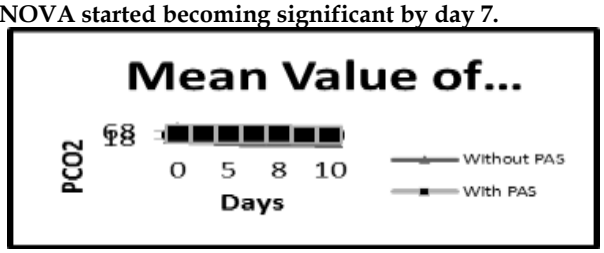

Figure-3: The mean values of PCO_2 in random donor platelet concentrates in two groups (with and without PAS) . The difference between two groups of repeated measurements using ANOVA was highly significant by day 3.

Discussion

The present study was done to assess different parameters of platelets stored in plasma and PAS like pH, glucose, LDH, PO_2 and PCO_2.Basis for including these parameters in this study is that there are two pathways through which platelets generate energy. One is Tri-carboxylic acid cycle (85%) and other is glycolysis (15%).[6]According to the recommendations of American Association of Blood Banks, the pH level of > 6.2 is an essential requirement for the quality control of blood components.[12] In present study pH of platelet concentrates stored in PAS was well

maintained till day 10 while the pH of platelets in plasma dropped rapidly after day 5 (p-< 0.001).

The PAS Plasmalyte- A used in this study contains acetate which acts as a buffer. During storage platelets use the anaerobic pathway of glycolysis and oxidative pathway of tri-carboxylic acid cycle for energy production.Major substrate for TCA pathway is free fatty acids(FFA). As PAS containing platelet concentrates contains less plasma, only small amount of FFA would be available for platelet metabolism. In this study the PAS contains acetate which is used as a substrate for platelet metabolism. By the formation of bicarbonate from the carbon dioxide produced by the acetate, very stable pH levels are maintained during platelet storage.[13] Our results are consistent with the findings of two other groups.[14,15] In conflict, studies carried out by some other groups showed a rapid fall in pH due to the very limited buffering capacity of Composol PAS.[16]

Glucose levels decreased during storage in both groups but this decrease was seen more in plasma (p- ≤ 0.001).Studies on PAS showed that presence of glucose is crucial for platelet metabolism.[5,17] LDH levels were raised in both groups but the rise was slightly more in plasma. The difference between the groups was not statistically significant. The buffering capacity of other platelet additive solutions (Composol) is approximately half to that of plasma, so they are more susceptible to the production of LDH by the platelets.[13] In this study, acetate,present in the PAS- Plasmalyte A, was found to be able to reduce LDH formation.[18,19] The difference in the results of various researches probably originated from the different constituents of the PAS used. [19]

The oxygen levels rose and carbon dioxide levels decreased during storage in both groups but these changes were seen early in plasma than in PAS indicating that oxygen consumption was reduced along with production of carbon dioxide (p- < 0.001). In another study, during 12 days storage of platelets in plasma and PAS similar results were reported.[5]

This study showed that all the parameters of stored platelets were well preserved in PAS but real effect of PAS on platelets can be uncovered only in human body. The platelets can be stored only at room temperature and so there is a risk of bacterial contamination. Further studies are required to overcome these problems.

Conclusion

PAS could replace plasma as a storage media for platelet concentrates to increase the life span of platelets. All the in vitro parameters were well preserved in platelet concentrates stored in PAS than in plasma.

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