

Trehalose An Additive Solution for Platelet Concentrate to Protect Platelets from Apoptosis and Clearance during Their Storage at 4°C

Vahid Baghdadi, Ph.D.¹, Reza Ranjbaran, Ph.D.², Fatemeh Yari, Ph.D.^{1*}, Mohammad Hessam Rafiee, Ph.D.¹

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
2. Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding Address: P.O.Box: 14665-1157, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
Email: f.yari@ibto.ir

Received: 02/December/2020, Accepted: 11/May/2021

Abstract

Objective: Although cold storage of platelets (PLTs) could decrease the risk of bacterial growth, it could affect on the PLTs viability and hemostatic function. At cold temperatures, trehalose can be used to substitute water, inhibit the solid-liquid transition phase of the PLT membrane, and stop Glycoprotein Iba (GPIba) polymerization. In this study, we evaluated the potential of trehalose for reducing the negative effects of cold storage on the apoptosis and the clearance rates of PLTs after long-term storage at cold.

Materials and Methods: In this experimental study, PLT concentrates (PCs) were maintained for five days in the different circumstances. PLTs were subsequently counted by using an automated hematology analyzer. Also water-soluble tetrazolium salt (WST-1) assay was performed to estimate the viability of PLTs. The activity of lactate dehydrogenase enzyme (LDH) was determined by a biochemical analyzer. And human active caspase-3 levels were measured by using enzyme-linked immunosorbent assay (ELISA) method. Also, we applied flow cytometry technique.

Results: PLTs count and viability were higher, while LDH amount was lower in trehalose-treated PLTs when compared with two other groups (P=0.03). The highest increase in the amount of caspase-3 levels in the PLTs was observed at 4°C. However, trehalose-treated and 4°C PLTs had a lower amount of active caspase-3 in comparison with 4°C PLTs. The level of PS expression on PLTs was lower in the trehalose-treated PLTs in compared with the two other groups (P=0.03). PLTs ingestion by HepG2 cells was enhanced in the 4°C-stored PLTs. However, the ingestion rate was significantly reduced in the trehalose-treated PLTs on day 5 of storage (P=0.03).

Conclusion: Trehalose can moderate the effects of cold temperature on the apoptosis, viability, and the survival rate of PLTs. It also decreases the ingestion rate of refrigerated PLTs *in vitro*.

Keywords: Cold Storage, HepG2, Platelet Transfusion, Trehalose

Cell Journal (Yakhteh), Vol 24, No 2, February 2022, Pages: 69-75

Citation: Baghdadi V, Ranjbaran R, Yari F, Rafiee MH. Trehalose an additive solution for platelet concentrate to protect platelets from apoptosis and clearance during their storage at 4°C. Cell J. 2022; 24(2): 69-75. doi: 10.22074/cellj.2022.7886.

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Introduction

Today, platelet (PLT) concentrates can be stored at 22°C for less than five days before transfusion (1). This short shelf life is because of the increase in bacterial contamination risk (2, 3). Another significant issue is correlated with the room temperature storage of PLTs' that resulted in the PLTs' loss of function and PLTs' structure changes, what is referred to the PLT storage lesion (PSL) (4, 5). Cold storage of PLTs can resolve several issues associated with 22°C- storage of them (6). Investigations have shown that cold storage of PLTs decreases the chance of bacterial contamination, reduces PLT metabolic activity, and minimizes the release of biological response modifiers (7-9). Many *in vivo* studies demonstrated that cold stored PLTs have the better function in the decreasing the bleeding time of patients with thrombocytopenia and functional disorders when in comparison with room temperature kept PLTs (10). However, due to the rapid removal of PLTs from the patients' bloodstream, use of cold-stored PLTs has been stopped since the 1970s (11, 12). Studies have indicated that storage of PLTs at a cold temperature for short-term (<4 hours) leads to clustering of GPIb receptors. Thereupon, and $\beta 2$ integrins on the hepatic macrophages

(Kupffer cells) selectively recognize uncovered β GlcNAc on the glycans within GPIb (13, 14). Moreover, prolonged cold storage of PLTs (48 hours at 4°C) has resulted in many changes such as increased galactose-terminated glycans on the GPIb, which are bound to the asialoglycoprotein receptors on the hepatocytes and thereby mediate PLTs clearance (15-17).

New methods may help to prevent PLT phagocytosis and protect the functional activity of refrigerated PLTs. Trehalose is a non-reducing disaccharide that is found in large amount in the nature. Some bacteria, fungi, plants and invertebrate animals produce it as a source of energy.

Trehalose could preserve phospholipids, proteins, and cells from damage (18). Trehalose is extensively utilized as a biomacromolecular protective agent, which is decomposed into the two molecules of glucose (GLU) and has no side or toxic effects (19). At low temperatures, trehalose can be used to replace water, prevent the solid-liquid transition phase of the PLT membrane, and halt GPIba polymerization. Hence, Trehalose is considered a satisfactory, potential, and protective agent for PLTs low temperature

storage (20). This sugar has been used as a cryoprotectant to stabilize PLT membranes through lyophilization. In the presence of Trehalose, lyophilized PLTs showed a longer shelf life and a better recovery upon restoration (21-23).

Recent investigations revealed that Trehalose could inhibit phagocytosis of cold temperature storage-PLTs by THP-1 cells (24). However, the impact of Trehalose on the phagocytosis and clearance of long-term stored-PLTs has remained unclear. Accordingly, in this study, we evaluated the effect of Trehalose on the ingestion rate of long-term stored-PLTs by HepG2 cells as a model for hepatocyte cells *in vitro*. Also, the survival and apoptosis rates of PLTs were also analyzed to evaluate the effects of Trehalose on the PLTs stored at cold.

Materials and Methods

Collection and preparation of platelet concentrates

This experimental investigation was confirmed by the College's Bioethics Ethics Committee (IR.TMI.REC.1396.004). In this study, PLT concentrates (PC) bags (24 bags) (Macopharma, France) with sodium citrate anticoagulant were collected by the Tehran Blood Transfusion Center (TBTC), Tehran, Iran.

Different parameters including, PLT enumeration, mean PLT volume (MPV), PLT distribution width (PDW), lactate dehydrogenase (LDH), water-soluble tetrazolium salts (WST-1), human active caspase-3, phosphatidylserine (PS), and the ingestion rate of PLTs by HepG2 cells were evaluated before adding Trehalose to the bags. Subsequently, utilizing a digital balance (Sartorius, Germany) and a Terumo Sterile Connecting Device (TSCD - II, Terumo Tubing welder, Japan), each PC bag was divided into three equal parts. Trehalose (Sigma-Aldrich, USA) with a concentration of 40 mg/ml was added to one of the bags. The Trehalose-containing bag and the control bag (without Trehalose) were transported to the refrigerator (4°C) while the bag without Trehalose (third bag) was kept at 22°C in a shaker- incubator. Since the usual storage temperature for PLTs is 22°C, one of the controls was kept at 22°C. It is worth mentioning that the second control bags, (4°C+without Trehalose), and third control bags, (22°C), did not receive any concentration of Trehalose.

Determination of the effective Trehalose concentration

Saccharomyces cerevisiae-derived Trehalose was purchased from Sigma-Aldrich, USA. Trehalose powder was mixed with saline, and several concentrations of Trehalose (20, 30, 40, 50, and 60 mg/ml) were added to PC bags. The PC bags were subsequently stored at 4°C for five days without agitation. The aforementioned PLT parameters were determined during the 5-day storage of PLT concentrates.

Determination of PLT count, MPV and PDW

PCs were diluted in the phosphate-buffered saline (PBS, M.P. Biomedicals, LLC, 1:2 dilution) and subsequently applied for assessment of PLT count, MPV, and PDW by

an automated hematology analyzer (Sysmex XT-2000i, Kobe, Japan).

Evaluation of platelet bags for bacterial contamination

To examine probable bacterial contamination on day 1, the samples of all the bags were placed in a 37°C incubator in the Thioglycollate medium for one week. Then all samples were cultured on the blood agar.

Assessment of the PLT metabolic activity using WST-1 assay

WST-1 cell proliferation assay kit (WST-1, Cayman, USA) was used to measure the activity of cellular mitochondrial dehydrogenases in the PLTs. In this test, the tetrazolium salt is changed to formazan by viable PLTs; therefore, the result indicates PLTs viability rate. Following diluted with PBS, 10×10^6 PLTs (100 μ l) were added into each well. Accordingly, 10 μ l of the WST-1 mixture was added to each well, and the plate was incubated at 37°C in an incubator for 4 hours. The absorbance of the samples was measured at 450 nm in a microplate reader (Asys Expert 96, UK).

Lactate dehydrogenase measurement

LDH enzyme was used as a PLTs lysis marker. Utilizing the pyruvate-lactate method, the LDH enzyme levels were measured at 340 nm through a biochemical analyzer (Hitachi 911, Japan) and LDH kit (Pars Azmoon, Iran). The results of this parameter were analyzed by using the relative standard curve method.

Human active caspase-3 evaluation

The human active Caspase-3 level was evaluated with an enzyme-linked immunosorbent assay kit (Invitrogen, USA). The kit sensitivity was 1.25 ng/ml. Based on the producer instructions, cell extraction buffer was mixed with 5×10^8 PLTs and then washed three times with the PBS. The cell extraction buffer was then added to the pellet and suspensions were incubated at room temperature for 15 minutes. After centrifugation at 4000g for 10 minutes, the supernatant was collected in a clean tube. The ELISA steps were performed according to the kit instructions. After completing the reactions, the optical density of the each sample was read at 450 nm and the concentration of the samples as well as controls was ultimately determined by using the standard curve of the kit.

Evaluation of phosphatidylserine surface exposure

Using Annexin V-FITC (Fluorescein isothiocyanate) assay kit (Biolegend, USA) the surface exposure level of PS was determined. Briefly, 1.5×10^6 PLT cells were incubated in the 300 μ l of annexin V binding buffer. Adding 5 μ l of FITC-labeled annexin V all samples were incubated at 22°C for 20 minutes. Using the CyFlow Space (Partec, Germany) all samples were evaluated by flow cytometry technique.

Preparation of mepacrine-labeled platelets

Mepacrine (Sigma-Aldrich, USA) is an Acridine derivative whose emission wavelength is within the range of FITC. PLTs labeling, 20 μ l of 20 mg/mL mepacrine was added to the 5×10^7 PLTs to 30 μ l PBS solution and incubated for 30 minutes at ambient temperature. Afterward, the PLTs were washed three times with PBS by centrifugation at 1200 g for 15 minutes. Ultimately, PLTs were prepared for adding to cultured HepG2 cells.

Ingestion of platelets by HepG2 cells *in vitro*

HepG2 cells (IBRC, Iran) were cultured in DMEM-F12 medium (Sigma, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, US). After the growth of the HepG2 cells, they incubated for 30 minutes in a serum-free medium. Then, mepacrine-labeled PLTs (5×10^7) were added to the each wells and incubated at 37°C for 30 minutes. Subsequently, the wells were washed three times with PBS, and HepG2 cells were detached from the culture plates by treatment with trypsin at 37°C for 2 minutes. The ingestion of mepacrine-labeled PLTs by HepG2 cells was evaluated by flow cytometry technique. HepG2 cells containing the ingested PLTs were identified by their green fluorescence related to mepacrine, and the PLT adherence to HepG2 cells was differentiated from ingested PLTs through PE- labeled anti-CD42b.

Statistical analysis

All data were statistically analyzed and processed by using commercially available SPSS software (Version 22, IBM Corporation, USA). Statistical analysis was performed by Paired t test. $P < 0.05$ were considered significant.

Results

Determining the effective dose of Trehalose

The best dose of Trehalose was 40 mg/ml. In this dose, PS exposure was less on the PLTs at all days of storage in comparison to other doses. Also, according to the WST-1 assay, higher viability of PLTs was obtained at this dose of Trehalose (Table 1).

Platelets count, size and width distribution

The count of PLTs reduced in all the study groups. The rate of decrease in the PLTs count was less in the presence of Trehalose. During storage, the order of PLTs count was as follows: Trehalose treated PLTs (4°C) > Non-treated PLTs (4°C) > Non-treated PLTs (22°C). The differences between Trehalose-treated PLTs (4°C) and non-treated PLTs (4°C) were not statistically significant, whereas, the differences between non-treated PLTs (4°C) and (22°C) were statistically significant (day 3, $P=0.03$, and day 5, $P=0.01$, Fig.1, Table 2).

MPV and PDW parameters significantly raised in the non-treated PLTs (4°C) group and Trehalose-treated (4°C) group in compared with the non-treated PLTs (22°C) group. The differences between Trehalose-treated PLTs (4°C) and non-

treated PLTs (4°C) groups were statistically significant on the fifth day of storage ($P=0.03$). At the 22°C, however, the mean of MPV significantly reduced on the both days (3 and 5 days) of storage in comparison with the first day ($P < 0.05$, Fig.1, Table 2).

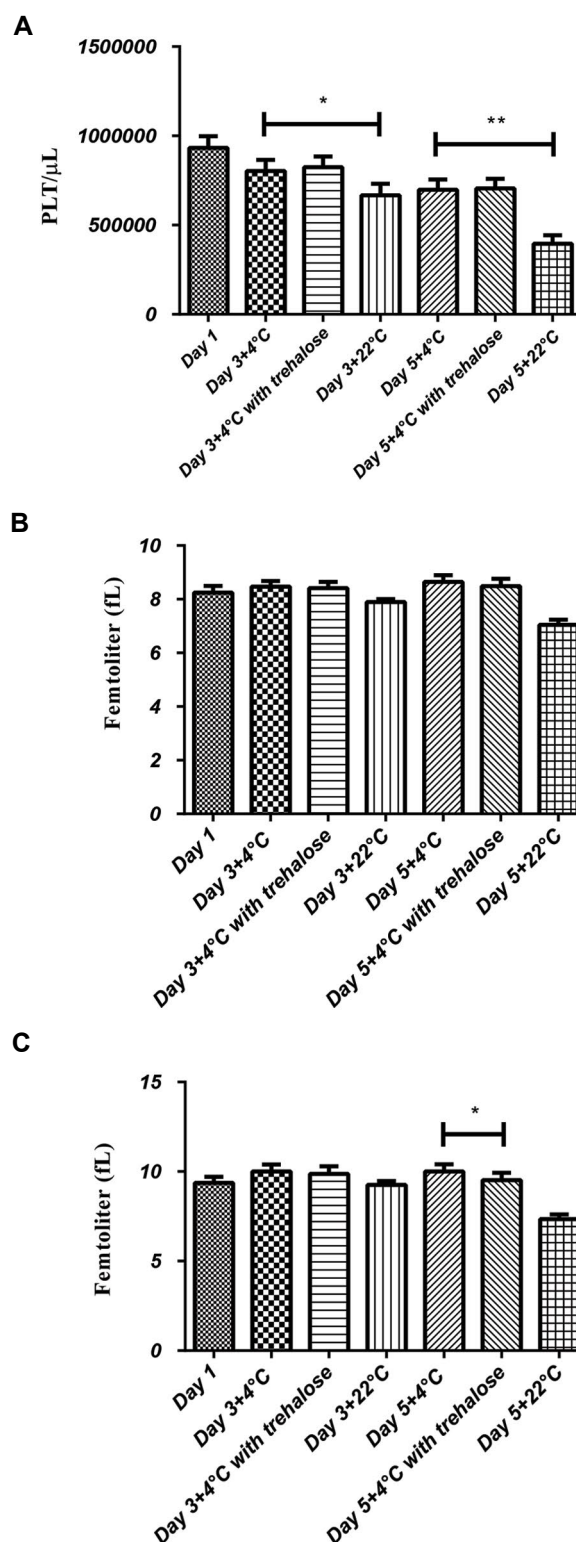


Fig.1: Comparative effect of cold, room temperature, and Trehalose on the PLTs count, MPV, and PDW. **A.** The count of PLTs was reduced during stored in all groups of investigation. Only, the differences between 4°C PLTs and 22°C PLTs were statistically significant (day 3, $P=0.03$, and day 5, $P=0.01$). **B.** Different amount of MPV. **C.** Different amount of PDW. The differences between Trehalose (4°C) PLTs and 4°C PLTs were statistically significant on the fifth day of storage ($P=0.03$). PLTs; Platelets, MPV; Mean PLT volume, PDW; PLT distribution width, *, $P=0.03$, and **, $P=0.01$.

Effects of Trehalose on The Viability of Platelets

Table 1: Determine the effective Trehalose concentration

Trehalose concentration (mg/ml) n=4	Storage days	Platelet count (10 ³ /μL)		MPV (fL)		PDW (fL)		Phosphatidyl-serine (%)		WST-1 (OD ₄₅₀ nm)	
		4°C	4°C+trehalose	4°C	4°C+trehalose	4°C	4°C+trehalose	4°C	4°C+trehalose	4°C	4°C+trehalose
20	3	685 ± 101	714 ± 111	7.9 ± 0.908	7.7 ± 0.945	8.2 ± 0.866	8.1 ± 0.901	25.9 ± 3.92	24.5 ± 3.17	0.285 ± 0.063	0.252 ± 0.059
	5	720 ± 193	788 ± 149	8.2 ± 0.918	8 ± 0.961	8.3 ± 0.958	8 ± 0.983	13.2 ± 3.72	11.6 ± 2.75	0.324 ± 0.096	0.349 ± 0.103
30	3	675 ± 109	685 ± 118	7.9 ± 0.752	7.8 ± 0.805	8.2 ± 0.784	8.1 ± 0.815	19.8 ± 4.15	17 ± 3.64	0.295 ± 0.084	0.314 ± 0.095
	5	705 ± 122	781 ± 133	9.7 ± 0.958	9.5 ± 0.999	10.8 ± 0.805	10.7 ± 0.819	12.9 ± 3.01	9.2 ± 2.65*	0.684 ± 0.213	0.874 ± 0.169*
40	3	669 ± 99	975 ± 121*	9.4 ± 0.837	9.3 ± 0.858	10.5 ± 0.818	10.3 ± 0.858	20.8 ± 4.75	11.7 ± 3.01*	0.512 ± 0.145	0.800 ± 0.141*
	5	725 ± 135	915 ± 141*	8.1 ± 0.901	7.9 ± 0.927	8.5 ± 0.912	8.3 ± 0.939	11.4 ± 3.28	9.7 ± 2.55*	0.519 ± 0.139	0.608 ± 0.128*
50	3	725 ± 97	747 ± 82	7.9 ± 0.799	7.7 ± 0.817	8.2 ± 0.738	8.1 ± 0.768	26.8 ± 3.95	24.7 ± 3.32	0.342 ± 0.103	0.380 ± 0.088
	5	842 ± 105	856 ± 93	8.4 ± 0.808	8.2 ± 0.818	8.7 ± 0.795	8.6 ± 0.804	19 ± 2.96	17.2 ± 3.10*	0.459 ± 0.111	0.526 ± 0.111*
60	3	682 ± 117	703 ± 101	8.1 ± 0.933	7.9 ± 0.957	7.9 ± 0.902	7.7 ± 0.937	31.5 ± 4.05	28.9 ± 3.88*	0.301 ± 0.097	0.352 ± 0.102
	5	685 ± 125	714 ± 121	7.9 ± 0.989	7.7 ± 0.991	8.2 ± 0.967	8.1 ± 0.998	25.9 ± 3.54	24.5 ± 3.67	0.285 ± 0.072	0.252 ± 0.069

Data are presented as mean ± SD. All data were statistically analyzed and processed by using commercially available SPSS software (version 22). MPV; Mean platelet volume, PDW; Platelet distribution width, and WST1; Water soluble tetrazolium salt, and *; P=0.03.

Table 2: The mean ± standard deviation for different parameters of platelets during the storage at different days and conditions

Study variables (n=12)	Day 1	Day 3 (4°C)	Day 3 (4°C+Trehalose)	Day 3 (22°C)	Day 5 (4°C)	Day 5 (4°C+Trehalose)	Day 5 (22°C)
Platelet count (10 ³ /μL)	933 ± 240	803 ± 234	823 ± 141	667 ± 240	697 ± 216	705 ± 202	396 ± 180
MPV (fL)	8.24 ± 0.958	8.47 ± 0.785	8.41 ± 0.880	7.89 ± 0.402	8.64 ± 0.951	8.48 ± 1.037	7.04 ± 0.731
PDW (fL)	9.36 ± 1.295	10 ± 1.454	9.87 ± 1.581	9.25 ± 0.810	10 ± 1.488	9.52 ± 1.533	7.34 ± 0.985
LDH (U/L)	295 ± 42.10	394 ± 47.53	352 ± 54.64	1990 ± 499	485 ± 40.89	439 ± 55.52	3087 ± 635
WST1 (OD 450 nm)	0.718 ± 0.256	0.594 ± 0.281	0.631 ± 0.274	0.26 ± 0.939	0.438 ± 0.81	0.504 ± 0.113	0.221 ± 0.74
Phosphatidylserine (%)	5.33 ± 2.22	10.69 ± 3.75	9.53 ± 3.84	54.79 ± 16.09	19.91 ± 5.22	16.82 ± 7.05	75.83 ± 7.30
active caspase-3 (ng/ml)	0.579 ± 0.172	1.657 ± 0.492	1.377 ± 0.668	0.958 ± 0.538	2.181 ± 0.722	1.930 ± 0.586	1.267 ± 0.593
HepG2 ingest (%)	19.79 ± 15.45	36.32 ± 18.01	38.61 ± 23.33	31.71 ± 20.85	37.58 ± 15.88	30.33 ± 12.06	32.35 ± 12.10

MPV; Mean platelet volume, PDW; Platelet distribution width, LDH; Lactate dehydrogenase, and WST1; Water soluble tetrazolium salt.

Evaluation of platelet bags for bacterial contamination

The results of bacterial culture on the Thioglycollate and Blood agar media were evaluated showed no effect of contamination.

The metabolic activity of platelets by WST-1 assay

The metabolic activity was proportional to the PLTs viability. It was diminished during storage in the all groups, and the highest decrease was observed in the PLTs storage at 22°C. The metabolic activity of PLTs was well maintained in the presence of Trehalose (4°C) compared with 4°C-kept PLTs, and the differences were statistically significant (P=0.03, Fig.2, Table 2).

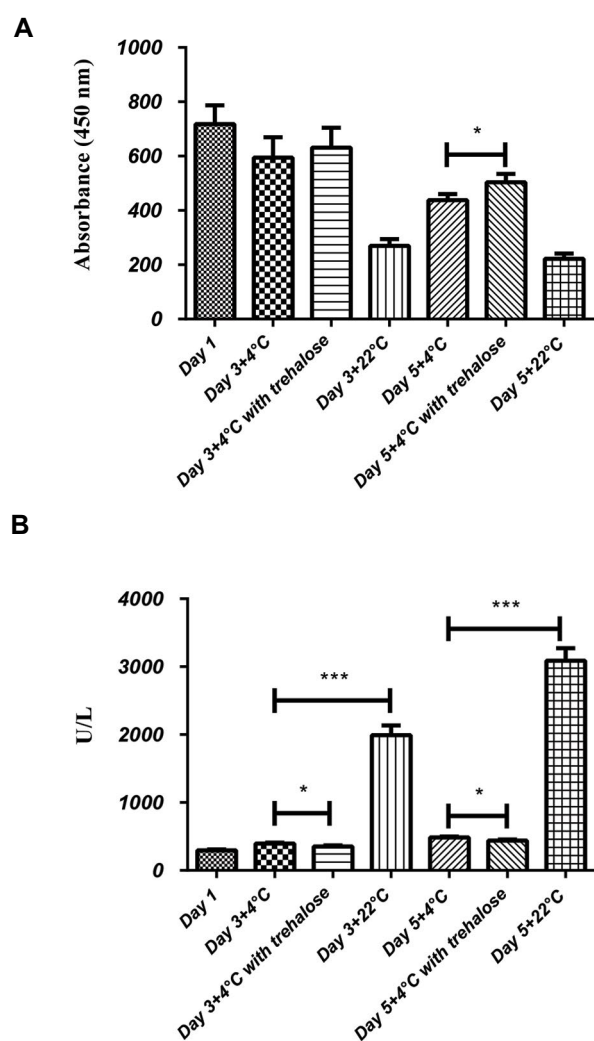


Fig.2: Effect of cold, room temperature and Trehalose on the lactate dehydrogenase (LDH) activity and the metabolic activity and the survival (WST1) amount of the platelets in different days of storage. **A.** The differences Wst1 results between Trehalose (4°C) PLTs and 4°C PLTs were statistically significant (P=0.03). **B.** LDH level was higher at room temperature (22°C) in comparison with 4°C (P<0.05). LDH in the PC bags was lower in the presence of Trehalose at 4 °C; the difference with 4°C groups was statistically significant (P=0.03). *, P=0.03 and ***, P=0.001.

The platelets lactate dehydrogenase levels

The LDH value of PC bags was increased during

storage in the all groups, and it was significantly higher at room temperature (22°C) in comparison with non-treated PLTs (4°C) and Trehalose-treated (4°C) groups (P=0.001). It is important to regard that the lowest value of LDH was observed in the Trehalose-treated PLTs (4°C) and the difference was statistically significant in comparison with non-treated PLTs storage at 4°C (P=0.03, Fig.2, Table 2).

Human active caspase-3 levels in the platelets

The level of human active caspase-3 was increased in the PLTs in all groups. Also, a higher increase was observed in the non-treated PLTs (4°C) group. There was a significant difference in active caspase-3 levels between PLTs stored at 4°C in the presence and absence of Trehalose on the fifth day of storage (P=0.03, Fig.3, Table 2).

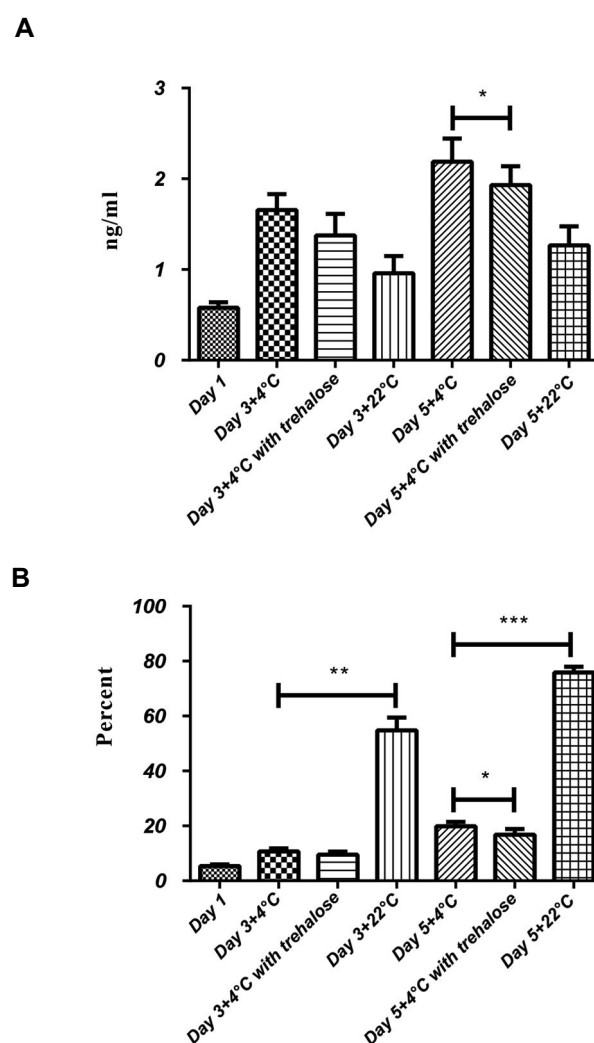


Fig.3: Effect of the temperature and Trehalose presence on the active caspase-3 levels and the exposure level of PS in the platelets during storage. **A.** There was a significant difference in active caspase-3 levels between 4°C PLTs stored with and without Trehalose at the fifth day of storage (P=0.03). **B.** The exposure level of PS was significantly lower in the presence of Trehalose (4°C) on day 5 in comparison with 4°C groups (P=0.03). PS; Phosphatidylserine, PLTs; Platelets, *, P=0.03, **, P=0.01, and ***, P=0.001.

The exposure level of phosphatidylserine on the platelets

During the storage time, the surface exposure of PS increased in the all groups. The level of PS exposure was significantly lower in the Trehalose-treated PLTs (4°C) on the 5th day in comparison with the non-treated PLTs (4°C, $P=0.03$). Also, the differences in the PS exposure between PLTs storage at 22°C group and other groups were statistically significant ($P=0.001$, Fig.3, Table 2).

Ingestion of the refrigerated platelets by HepG2 cells

PLTs storage at cold temperature caused an increase in the PLTs ingestion rate by HepG2 cells in comparison with PLTs stored in 22°C during 5-day storage. Trehalose caused a lower clearance rate for 4°C storage PLTs by HepG2 cells in compared with 4°C storage PLTs in the absence of Trehalose, and the differences were significant on the fifth day of storage ($P=0.03$, Fig.4, Table 2).

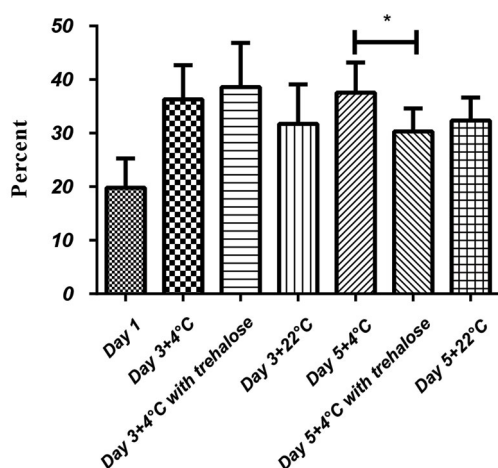


Fig.4: Trehalose effect on the ingestion of platelets by HepG2 cells during storage in the different groups of study [22°C, non-treated PLTs (4°C) and Trehalose-treated (4°C)]. The differences were significant at the fifth day of storage ($P=0.03$). *, $P=0.03$.

Discussion

In this investigation, the impact of Trehalose on the viability, apoptosis, and the clearance rate of PLTs was evaluated during 5-day PCs storage. We found that Trehalose could provide protective effects on the 4°C-storage PLTs. Trehalose could reduce the ingestion rate of the refrigerated PLTs by HepG2 cells through preventing PS exposure and caspase-3 activity. It was also able to keep better the viability and metabolic activity of the refrigerated PLTs.

Based on the results of this study, PLTs enumeration reduced during storage in the all of our groups. However, the highest reduction in the PLTs counts was observed in the PLTs stored at 22°C. In Trehalose-treated PLTs, the PLTs counts were higher and better preserved than

our other groups. However, the differences were not statistically significant. The results of this study were consistent with the results reported in our previous research as well as findings published by Handigund et al. (25-27). They showed that PLTs count diminished in both room temperature and 4°C during storage (25). Additionally, it was in line with the results obtained by Wang et al. which showed PLTs treated with PLT additive solution and Trehalose had a high count during storage at 10°C (28).

According to this study, PLT survival decreased during storage. The lowest viability was observed in the PLTs maintained at room temperature, whereas the highest viability was seen in the Trehalose-treated PLTs (4°C). The results of this investigation showed that Trehalose could preserve the survival rate of PLTs.

Additionally, in this study, we indicated that the LDH activity was lower in Trehalose-treated PLTs (4°C) compared to non-treated PLTs (4°C) and room temperature (22°C) PLTs. The highest enzyme activity was observed in PLTs maintained at 22°C on the both days (3 and 5 days) of storage. One of the essential agents in preserving the quality and survival of PLTs in PCs is the low amount of LDH released into the bag during storage (29). The results of this study are consistent with findings of Dasgupta et al. (30) research regarding the effects of cold temperature and Trehalose on the reducing LDH levels in the stored PLTs.

As Marini et al. (12) reported, PS exposure on PLTs is an essential indicator of apoptosis. The result of this study showed that exposure of PS in the Trehalose-treated PLTs was lower than those of other groups of study. In consistent with our findings, Liu et al. (24) demonstrated that Trehalose could reduce PS expression levels on the PLTs when maintained at low temperature.

This study also showed an increase in the caspase-3 levels in the all groups during the 5-day storage of PLTs. This finding corroborates the results obtained by Quach et al. (29) and Perrotta et al. (31). In the PLTs treated with Trehalose, the level of caspase-3 was lower than other groups. These results were also consistent with the findings of Liu et al. (24).

We also investigated the effect of Trehalose and cold storage on the ingestion rate of PLTs by HepG2 cells. According to our findings, PLTs ingestion increased in the all groups during storage. However, the lowest increase in the PLT ingestion was related to the PLTs stored at 22°C. This finding was consistent with the previous reports (14, 32). We observed an increase in the PLTs ingestion by HepG2 cells during 5-day storage in the cold temperature.

However, the Trehalose-treated PLTs had a lower uptake than the non-treated PLTs. This might be due to the protective effect of Trehalose on the phospholipids, proteins, and cells against cold storage-mediated damages (18).

Our results were in line with the study of Liu and co-workers (24). Although Liu et al. stored PLTs for a short period in the cold temperature, they showed that removal of PLTs by THP1 cells reduced in the presence of Trehalose. These findings support the protective effects of Trehalose for the PLTs in the PCs during storage at 4°C.

Conclusion

Trehalose could moderate the effects of cold temperature on the apoptosis and survival of PLTs. It also decreased the ingestion rate of long-time refrigerated PLTs *in vitro*. Further studies with more sample numbers are required to demonstrate the effect of trehalose on the reducing the clearance rate of PLTs.

Acknowledgments

This study was the result of a Ph.D. thesis, financially supported by Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Iranian Blood Transfusion Organization, Tehran, Iran. The authors have disclosed no conflicts of interest.

Authors' Contributions

V.B.; Performed all of experimental work, contributed to the design of the study, interpretation and manuscript drafting and reviewing as a Ph.D. student. R.R.; Contributed to data and statistical analysis, and interpretation of data. F.Y.; Was responsible for overall supervision of the Ph.D. thesis and contributed to the study conception and design. M.H.R.; Contributed to conception and design. All authors read and approved the final manuscript.

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