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ORIGINAL ARTICLE

L-carnitine effectively improves the metabolism and quality of platelet concentrates during storage

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Abstract Human platelets undergo structural and biochemical alternations during storage which are collectively called platelet storage lesion (PSL). PSL is characterized as metabolic and functionally changes. It causes decrease in platelet recovery and survival. Here, we evaluated the effect of L-carnitine (LC) on the metabolism, function, and mitochondrial metabolic activity of platelet during storage. Platelet-rich plasma was used to prepare platelet concentrate (PC) in Iranian Blood Transfusion Organization. For this purpose, ten PC bags from healthy donors were stored at 22 °C with gentle agitation in the presence or absence of LC. The effects of LC (15 mM) on the platelet quality were assessed by analyzing the levels of glucose, lactate, ATP, and lactate dehydrogenase (LDH) activity. Platelet aggregations induced by arachidonate and ristocetin were analyzed by aggregometer. Platelet mitochondrial melablolic activity was measured by tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay; platelet count and mean platelet volume were also determined by a hematology analyzer during 5 days of PC storage. The results indicated that LC could significantly decrease lactate concentration and glucose consumption accompanied with the increased oxygen consumption in stored PC. LDH activity also less significantly increased in LC-treated PC on days 2 and 5 of storage. Platelet aggregation in response to the ristocetin and arachidonate was significantly higher in LC-treated PC than that in untreated PC on day 5 of storage. Finally, platelet mitochondrial metabolic activity less significantly decreased in LC-treated PC compared to the control group on days 2 and 5 of storage. It seems that LC would be a good additive to reduce PSL and improve the platelet metabolism and quality of the stored PC for platelet transfusion therapy.

Keywords L-carnitine · Platelet quality · Stored platelet concentrates · Platelet storage lesion

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Introduction

Platelets play an important role in normal hemostasis by repairing breaks in the small blood vessel walls and releasing phospholipids and other release products that are required for in vivo hemostasis [1]. Platelets are transfused to patients who have very low platelet count with severe thrombocytopenia or to patients with platelet dysfunction to prevent bleeding or induce hemostasis. To ensure good hemostatic function in the recipient, it is important that the functionality of the platelets used for transfusion is well preserved. Platelet concentrate (PC) is recently stored for a maximum of 5–7 days due to the risk of bacterial growth [2]. Many factors influence the quality of platelets during storage including the preparation method [3], the material of the storage bag [4], the bag ability to gas exchange [3], and the storage temperature [5, 3]. During storage, platelets undergo numerous changes due to lactate accumulation and a decrease in pH [6]. There is a change in the functional integrity of the platelet [7, 8], a change in the shape of the platelet which may be responsible for exposure of phosphatidyl serine on the surface of the platelet and microvesiculation [9]. Other changes include release of granule content causing expression of CD62P on the platelet surface and decrease in agonist-induced aggregation. These progressive changes in platelet during storage are termed the platelet storage lesion (PSL) [6–12]. The biochemical

mechanisms responsible for the PSL are not fully understood, but metabolic disturbance and inadequate metabolic support play an important role in the loss of platelet viability in the PSL [13–16]. The pH condition has been shown to greatly affect the quality of the PC. A pH below 6.0 is associated with loss of platelet viability [4, 5]. PSL also correlates with reduced in vivo recovery and survival as well as hemostatic activity after transfusion [17, 18]. Resting platelets derive substantial energy from β-oxidation of fatty acids. In theory, fatty acid oxidation could maximize ATP production and decrease the need for ATP production through glycolysis, and hence, decreasing lactate production leads to prevent the fall in pH [19, 20]. During platelet storage, β-oxidation of long chain fatty acids is impaired, leading to an increase in glucose consumption, production of lactate, and decrease in pH [21]. L-carnitine (LC) or γ-trimethylamino-β-hydroxybutric acid (biologically active form of carnitine) is a small molecule found in almost all cells. LC is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine. Actually, synthetic LC is a white, highly water-soluble powder and is a substance with good thermostability (up to 200 °C). It has very low toxicity with an LD_{50} in rodents of 9 g/kg body weight. Ionizable groups (COO^- and N^+ $(CH_3)_3$) of LC are over 90 % dissociated at physiological pH (7.4) due to their P_K values [22–25]. This molecule is essential for the oxidation of fatty acids. LC acts by reacting with fatty acids and facilitates the transport of long chain fatty acid into the mitochondrial matrix, thereby enhancing β-oxidation that facilitates ATP formation, and it may affect the biological function of the platelet. Recent studies have shown that LC decrease glycolysis in liquid-stored platelet. In fact, LC may decrease glucose consumption, and lactate generation can improve in vitro function of the platelet. Also, LC modulates platelet activation through the antioxidant mechanism [26–29]. It seems that LC may be beneficial for platelet hemostatic function. However, there is limited information on the effect of LC on the metabolism and quality of PC during storage time. The aim of this study was to reduce PSL by adding LC that may affect on the function, metabolism, and mitochondrial metabolic activity of platelet compared to the untreated PC.

Materials and methods

Preparation and storage of PC

The present study was conducted on the samples of ten blood donors. The study was approved by the local ethical committee, and the informed consent was obtained from the blood candidates by Iranian Blood Transfusion Organization (IBTO). The random donor platelets were prepared by platelet-rich plasma (PRP) method in IBTO [30]. The entire PC was obtained from the whole blood by routine phlebotomy into 450-ml bags containing 63 ml of citrate phosphate dextrose adenine solution as anticoagulant in triple blood bags (JMS Ltd, Singapore). All of these PC were kept in platelet incubator at 22 °C and agitated constantly for 5 days.

Sample preparation

Ten single donor PC bags were randomly selected. Each bag included 40–60 ml PC with more than 1×10^9 PLT/ml. The protocol for the division of PC into two portions and addition of LC (Sigma-Aldrich, Germany) was as follows: On the day of PC collection (day 0), each PC bag (A) was connected to one transfer bag (B) using a connecting device instrument (TSCD-II, Terumo Sterile Tubing Welder, Japan). Half of the bag constituent was transferred to the transfer bag B via transfer tube using a digital balance (Sartorius, Germany).

All parameters were measured on the day 0 of PC collection in bags A and B (before treatment), and both bags were transferred to a shaker incubator at 20–24 °C. On the day after the sample collection (day 1), the powder of LC was dissolved in sterile normal saline (1,000 mM stock) and filtered using 0.22-μm filters (GVS, USA). For aseptic infusion of LC into bags (B), LC solution was added in a final volume of 1 ml using insulin syringe under class II laminar flow, and its final concentration was adjusted at 15 mM. Equal volume (1 ml) of sterile normal saline was added to bag (A) as a control. The concentration of 15 mM was chosen based on a pilot study. The case (B) and control bags (A) were stored up to 5 days at 20–24 °C under agitation in a shaker incubator for analyzing the parameters that could potentially affect the metabolism, function, and mitochondrial respiration of platelets in the both case and control groups.

Pilot study for the determination of the best and effective concentration of LC

Different concentrations of LC (5, 10, 15, and 25 mM) were used. The case and control bags ($n=5$) were stored up to 5 days at 20–24 °C under agitation in a shaker incubator. We evaluated the parameters of lactate, LDH, agonist-induced platelet aggregation, and mitochondrial metabolic activity in stored PC (both the case and control). Based on these results, we chose the most appropriate concentration of LC for the platelet quality during storage.

Platelet enumeration and MPV assay

Platelet counts and mean platelet volume (MPV) were determined by using an automated electronic particle counter (Sysmex, K-1000, Kobe, Japan). For this purpose, 0.5 ml of stored PC was diluted with the same volume of phosphate buffer saline (PBS).

Measurement of glucose, lactate, and lactate dehydrogenase activity

Glucose, lactate concentration, and LDH activity were measured by colorimetric methods using assay kits (all from Randox, UK) and with a Chemistry Analyzer (Hitachi 911, Japan). As the manufacture's instruction, for glucose and lactate measurement, the samples were incubated in the appropriate reaction mixture for 10 min at room temperature, and the absorbance was measured at 570 nm. Additionally, LDH activity was measured at 340 nm by using pyruvate-lactate method. The value of these parameters was assessed according to the standard curves.

Blood gas analysis and pH measurement

The pH value, amount of HCO3, and blood gases of pO2 and pCO_2 were immediately determined in stored PC using an automated blood gas analyzer (Jem-3000, USA).

Measurement of ATP in stored PC

ATP was measured in the stored PC by a colorimetric method (Abcam, Cambridge, UK) as below: 1 ml of stored PC (1×10^9 PLT/ml) was collected from the bags and frozen quickly in liquid nitrogen for 1 week before the experiment (freezing could prevent ATP depletion until the day of analysis). In the day of analysis, 400 μl of stored PC was mixed with 100 μl of ice-cold perchloric acid (4 M), vortexed briefly, and incubated on ice for 5 min. Then, centrifugation was carried out at 13,000*g* for 2 min at 4 °C and supernatant transferred to a fresh tube. Excess perchloric acid was neutralized by adding an equal volume of ice-cold 2 M KOH and vortexed briefly. The sample was again centrifuged at 13,000*g* for 15 min at 4 °C, and the supernatant was collected for ATP assay, briefly: 100 μl of ATP assay buffer was added to deproteinized stored PC and centrifuged at 15,000*g* at 4 °C for 2 min. Afterward, 50 μl of the supernatant was added to the wells of a microplate. Then, mixture of ATP probe, ATP converter, and developer mix was added to each well and mixed well and incubated at room temperature for 30 min. The optical density of each sample (prepared in duplicate) was measured at 570 nm using plate reader. Finally, the standard curve was drawn and ATP concentration calculated.

Agonist-induced platelet aggregation

Three milliliters of stored PC was divided into two tubes. One of the tubes was used to prepare platelet poor plasma (PPP). Platelet count was determined in the other tube and diluted to a final concentration of 3×10^5 PLT/μl using PPP. Afterward, 50 μl of ristocetin (Helena, France) and 50 μl of arachidonate (Helena, France) were added to 450 μl of diluted PC separately at the final concentration of 1.5 mg/ml for ristocetin and 0.5 mM for arachidonate. Agonist-induced platelet aggregation response was monitored optically on an aggregometer (Helena-Packs-4, France). Results were reported as the maximum change in light transmission (%) for a total time of 4 min.

Analysis of the platelet mitochondrial metabolic activity using MTT assay

In order to measure the mitochondrial metabolic activity of stored PC, the tetrazolium salt 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Roche, Germany) was used. The MTT assay is a suitable indicator of mitochondrial activity and in this method; NADPH-dependent mitochondrial oxidoreductase enzymes are capable of reducing the tetrazolium dye to insoluble formazan. The mitochondrial respiration rate was directly correlated with the amount of purple formazan crystals [31]. For this purpose, stored PC was diluted in PBS to a concentration of 3×10^5 platelets/μl. One hundred microliters of diluted platelets was incubated with 10 μl of MTT (5 mg/ml) for 4 h at 37 °C. After addition of 100 μl of lysis buffer (10 % SDS, 0.02 mol HCl/l) and incubation for overnight at 37 °C, the absorbance was measured at a wavelength of 570 nm by the spectrophotometer.

Statistical analysis

Data processing and analysis were performed using the SPSS v.18.0 (SPSS Inc., Chicago, IL, USA). Paired *t* test was performed for comparison between groups in each time point. To examine the interaction effect between storage day and different incubation media (control and treatment group), two-way repeated measure ANOVA with two within-subject factors (2 paired group×3 times) was conducted. Sphericity assumption was checked, and in the case of violation, *p* value of Wilks' Lambda multivariate test was reported. For more information about the interaction, contrasts were performed comparing storage days against the previous day within groups. Also, for post hoc multiple comparisons of times, Bonferroni correction has been applied. All *p* values less than 0.05 ($p<0.05$) were considered as significant.

Results

Results of the pilot study

The results of pilot study on the effects of different doses of LC (5, 10, 15, 25 mM) on PC during storage showed that the concentration of 15 mM of LC was the most effective on stored PC quality in comparison with the control group.

Particularly, LDH activity highly significantly decreased ($p<0.01$) in that dose of LC on day 5 of storage. Also, ristocetin highly significantly increased ($p<0.01$) as well, indicated better aggregation comparison with other doses of LC. According to MTT assay, mitochondrial metabolic activity showed less decrease at the dose of 15 mM LC-treated PC ($p<0.01$) compared to other doses of LC on day 5 of storage (data in details are shown in Table 1).

Results of the platelet count and MPV indexes in PC during storage

The levels of platelet count and the MPV in two groups are presented in Table 2. These parameters were both better maintained in LC-treated stored PC. According to the paired *t* test, platelet count showed less statistically significant decrease ($p=0.045$) in LC-treated stored PC compared to the control group on day 5 of storage. On the other hand, according to the two-way repeated measure ANOVA, the two groups had different effect on platelet count depending on the day of storage ($p=0.034$, interaction effect is shown in Table 2). For MPV, according to the paired *t* test, there were statistically significant differences between the two groups. MPV on the days 2 and 5 of storage significantly increased in the control group compared to LC-treated PC ($p=0.038$, $p=0.006$). According to the ANOVA, MPV increased statistically higher in the control group than LC-treated group from storage day 0 to 2 and days 3 to 5 ($p=0.038$, $p=0.047$) .

Results of the metabolic parameters in PC during storage

All results of the metabolic parameters in two groups including glucose, lactate, and ATP concentration, LDH activity, pO_2, pCO_2, HCO_3^-, and pH are presented in details in Table 2. Overall, the lactate concentration, LDH activity, and pO_2 in LC-treated PC compared to the control group showed less statistically significant increase especially on day 5 of storage. In contrast, glucose and HCO_3^- concentrations in LC-treated stored PC compared to the control group showed less statistically significant decrease especially on day 5 of PC storage. In case of glucose concentration, the paired *t* test analysis showed less significant decrease in LC-treated stored PC compared to the control group on day 5 of storage (327.6±47.32 and 344.7±45.6 mg/dl for the control and case, respectively) ($p=0.004$). Meanwhile, according to ANOVA analysis, glucose concentration in the control group showed more decrease compared to LC-treated stored PC (days 2 to 3) ($p=0.004$). According to the paired *t* test, a statistically significant difference was shown between the untreated and LC-treated PC for lactate concentration in each time point (days 2, 3, and 5 of storage, $p=0.006$, $p=0.002$, and $p=0.034$). On the other hand, two-way repeated measure ANOVA showed that both groups had different effect on lactate depending on the day of storage ($p=0.031$, interaction effect is shown in Table 2). Lactate showed more increase from days 0 to 2 ($p=0.016$) and from days 2 to 3 ($p=0.021$) of storage in the control group (according to the ANOVA). These results indicate that LC-treated PC had significantly lower lactate on all days compared to the control group (Fig. 1). LDH activity was significantly lower in LC-treated stored PC compared with the control group on the days 3 and 5 of storage ($p=0.013$ and $p=0.003$). ANOVA analysis showed that the trend of LDH changes was also significantly different between groups with the lower rate of elevation in LC-treated PC ($p=0.006$) (Table 2). The value of pH of stored PC was acceptable in both case and control samples. According to paired *t* test and also ANOVA analyses, there were no significant differences between the two groups of LC-treated and untreated stored PC on each storage day ($p=0.269$). However, pH values were better maintained in LC-treated stored PC. In case of pO2, the paired *t* test analysis showed more significant increase in the control stored PC compared to the LC-treated PC on the day 5 of storage (165.3±12.21 and 149.0±17.11 mmHg for the control and case, respectively) ($p=0.045$), and ANOVA analysis demonstrated significantly more increase in pO2 levels from days 2 to 5 of storage in the control group ($p=0.013$). Regarding pCO_2, there were no statistically significant differences between the two groups during storage according to paired *t* test. The results of pCO_2 indicate that there is no interaction effect between storage time and the two groups ($p=0.502$), according to ANOVA. Buffering status of stored PC, depending on HCO_3 concentration, was better maintained in LC-treated stored PC compared to the control group (Table 2). Paired *t* test showed that there was a statistically significant decrease in HCO_3 concentration in the control group compared to LC-treated stored PC on the day 5 of storage (4.71±1.03 and 5.96±1.15 mM for the control and case, respectively) ($p=0.013$), and ANOVA analysis indicated that HCO_3 concentration showed deeper decline in the control group during storage days 2 to 5 ($p=0.017$). Regarding ATP, both paired *t* test and ANOVA analyses showed that there were no statistically significant differences between ATP concentration in LC-treated stored PC and control group during storage (Table 2). However, it seemed that ATP values were better maintained in LC-treated stored PC.

Agonist-induced platelet aggregation

In platelet aggregation assay, the responses to agonists were better in LC-treated stored PC than the control group (Figs. 2 and 3). In this regard, paired *t* test showed that the response to the ristocetin was significantly higher in LC-treated stored PC compared to the control group during all the storage days ($p=0.001$, $p=0.006$, and $p=0.001$) (Fig. 2). The response to the ristocetin was the same as well, according to ANOVA analysis

Table 1 Results related to the effects of LC (5, 10, 15, and 25 mM) on PC as a case compared to control group at the days 2, 3, and 5 of storage (in pilot study)

	Day 1					Day 2		
	Control	5 mM LC	10 mM LC	15 mM LC	25 mM LC	Control	5 mM LC	10 mM LC
Lactate (mg/dl) X±SD, n=5	63±21	58±19**	56±20**	55±20**	56.1±17.8**	100±29	94±25**	91±24**
LDH (IU/l) X±SD, n=5	1,136±513	1,031±449	1,042±402	1,009±357*	998±364*	2,530±799	2,238±757*	2,201±781*
Arachidonate-induced aggregation (%) X±SD, n=5	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Ristocetin -induced aggregation (%) X±SD, n=5	[a]	[a]	[a]	[a]	[a]	43±8	46±5	45±7
MTT X±SD, n=5	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]

	Day 2		Day 3				
	15 mM LC	25 mM LC	Control	5 mM LC	10 mM LC	15 mM LC	25 mM LC
Lactate (mg/dl) X±SD, n=5	84±20**	90±23.5**	132±34	120±25**	117±25**	111±22**	116±25.1**
LDH (IU/l) X±SD, n=5	2,096±791*	2,284±918*	3,784±1,074	3,624±978*	3,570±1,008*	3,396±1,005**	3,503±1,188*
Arachidonate-induced aggregation (%) X±SD, n=5	[a]	[a]	10±2	25.7±11*	28±12*	29.6±7.8*	28.3±12.9*
Ristocetin -induced aggregation (%) X±SD, n=5	45±8	44.5±7	17±8	21±9*	23±6*	30.4±5.2**	19.35±6.83*
MTT X±SD, n=5	[a]	[a]	2.2±1	2.6±1*	2.7±1*	3.34±1**	2.9±1.15*

Differences were considered statistically significant at $p<0.05$* and $p<0.01$**

[a] This parameter was not measured in days 2 and 3

Table 2 Statistical comparisons of different parameters between LC-treated PC (case) and control group and between groups in each time point and over time (days 0, 2, 3, 5) are presented

Parameters, day of storage N=10	Control		Case		p Value[b]
	Mean	SD	Mean	SD	
PLT × 10^3/μl					0.034 [a]
0	1,223.35	373.26	1,229.52	369.98	0.535
2	928.53	213.72	942.33	238.36	0.447
3	816.82	213.26	845.46	219.89	0.815
5	627.27	189.57	693.52	228.29	0.045
MPV (fL)					0.001 [a]
0	8.48	0.38	8.50	0.38	0.443
2	8.56	0.56	8.41	0.63	0.038
3	8.67	0.55	8.51	0.51	0.061
5	8.74	0.49	8.27	0.44	0.006
Lactate (mg/dl)					0.031 [a]
0	52.30	18.01	51.92	18.55	0.536
2	62.61	23.30	55.34	19.53	0.006
3	89.10	23.10	75.40	17.15	0.002
5	122.30	34.38	102.30	26.39	0.034
Glucose (mg/dl)					0.029 [a]
0	473.40	37.27	472.40	37.41	0.293
2	407.90	36.73	401.80	30.17	0.299
3	358.30	40.29	376.40	38.54	0.073
5	327.60	47.32	344.70	45.61	0.004
LDH (IU/l)					0.006 [a]
0	582.90	71.24	589.90	86.07	0.573
2	1,379.10	517.65	1,178.00	538.44	0.099
3	2,542.40	304.46	2,087.70	566.64	0.013
5	3,774.60	903.93	3,259.20	1,009.50	0.003
pH					0.269 [a]
0	7.30	0.09	7.29	0.10	0.502
2	7.39	0.07	7.29	0.06	0.592
5	7.38	0.07	7.34	0.15	0.319
PO_2 (mmHg)					0.036 [a]
0	144.30	17.49	145.10	18.89	0.309
2	141.30	15.18	144.20	27.71	0.741
5	165.30	12.21	149.00	17.11	0.045
PCO_2 (mmHg)					0.502 [a]
0	40.05	10.84	40.26	11.25	0.366
2	22.20	2.94	22.80	3.16	0.658
5	9.70	2.45	11.40	3.72	0.165
HCO_3 (mmol/l)					0.017 [a]
0	14.81	1.77	14.98	1.80	0.127
2	13.54	1.21	13.57	1.27	0.921
5	4.71	1.03	5.96	1.15	0.013
ATP (nmol PLT × 10^9/ml)					0.524 [a]
0	7.68	0.42	7.66	0.30	0.794
2	6.1	0.40	6.62	0.39	0.195
3	5.93	0.37	6.02	0..26	0.836
5	4.80	0.23	4.77	0.45	1
Arachidonate (%)					0.002 [a]
0	83.86	4.05	83.41	4.84	0.540
2	68.03	7.33	71.61	7.37	0.05
3	28.98	13.59	43.60	12.97	0.001
5	15.61	6.48	25.25	11.06	0.001
Ristocetin (%)					0.002 [a]
0	83.80	5.07	84.10	5.02	0.730
2	69.32	4.98	75.16	5.50	0.001
3	50.27	7.29	54.78	7.49	0.006
5	21.47	5.66	33.47	6.75	0.001
MTT (OD at 575 nm)					<0.001 [a]
0	5.05	0.44	5.03	0.69	0.825
2	3.97	0.15	4.49	0.36	0.001
3	2.35	0.83	3.10	0.77	0.001
5	1.16	0.40	1.85	0.44	0.001

[a] p Value was reported for interaction between group and time from two-way repeated measure ANOVA

[b] p Value was reported for mean comparison between groups at each time point from paired t test

Differences were considered statistically significant at $p<0.05$

(p=0.002). In case of arachidonate, the response was higher in LC-treated stored PC compared to the control group (p=0.001 and p=0.001) on the days 3 and 5 of storage, according to paired t test (Fig. 3). ANOVA analysis indicated that the response to arachidonate was also significantly higher in LC-treated stored PC compared to the control group during all the storage days (p=0.002) (Table 2).

MTT assay

Platelet mitochondrial metabolic activity in stored PC was assessed by MTT method. Water-soluble formazan (MTT product, as the detectable factor in this assay) increased in the LC-treated stored PC compared to the control group. Our results indicated that in LC-treated PC, mitochondrial respiration is better preserved than the control group. Both paired t test and ANOVA analyses showed that the MTT product was more significant in LC-treated stored PC compared to the control group during days 2, 3, and 5 of storage (p=0.001, p=0.001, and p=0.001) (Fig. 4). Meanwhile, ANOVA analysis indicated that MTT product was also significantly higher from days 0 to 2 (p=0.001) and from days 2 to 3 (p=0.033) in LC-treated stored PC compared to the control group.

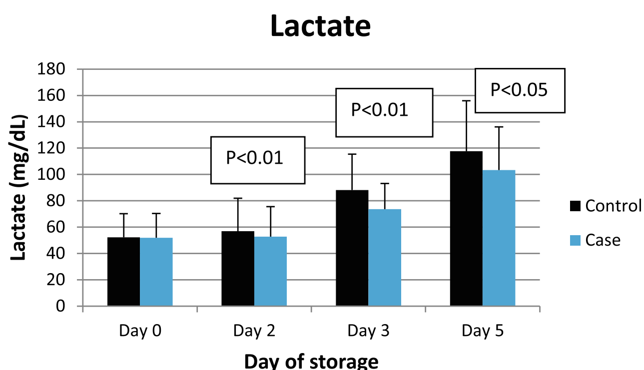

Fig. 1 Lactate concentration at different days of storage in LC-treated PC (case) and PC without LC (control)

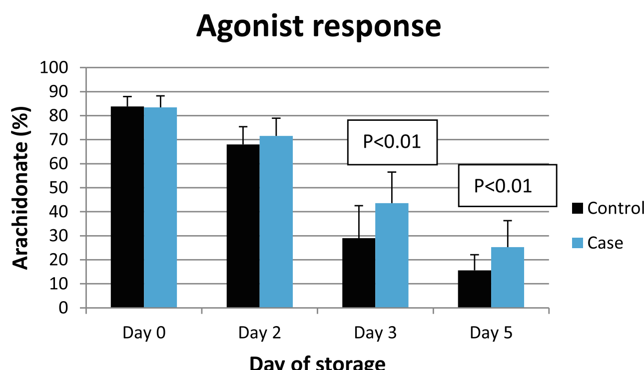

Fig. 3 Platelet aggregation in response to arachidonate at different days of storage in LC-treated PC (case) and PC without LC (control)

Discussion

PSL is, in fact, a complex process that is influenced by physical, chemical, and metabolic factors related to platelet preparation and storage which can reduce the quality of platelet before transfusion. For this reason, much research is being done in this area to understand the mechanism of PSL which could have important implications for improvement of platelet transfusion [10]. Platelets are morphologically, biochemically, and functionally changed during storage. Metabolic disturbance plays an important role in the loss of platelet viability in the PSL. Resting platelets derive substantial energy from β-oxidation of fatty acids. More than 80 % of ATP regeneration is based on oxidative phosphorylation, and free fatty acid (FFA) is the most important substrate used for ATP production [32]. During storage, the oxidation of long chain fatty acids is impaired, leading to increase in glucose consumption, production of lactate, and a decrease in pH [6, 33, 34, 35]. In fact, this event can lead to morphological changes correlated with decreased in in vivo viability and reduce survival of PC [10, 36, 37]. In this regard, finding the ways to reduce PSL and preserve quality of PC during storage is important for platelet transfusion. According to Gulliksson et al. [36], one of the ways to prolong the platelet life span was to reduce glucose consumption and decrease lactate accumulation. In consistent with this idea, we tried to plan a project to improve the quality of the PC during storage time using LC as a natural amino acid-derived molecule. Among the advantages of LC is its safety, being nontoxic and nonallergic [24]. For this purpose, in pilot study of this research, we found that the concentration of 15 mM of LC was the most effective on the stored PC quality, and we therefore used this amount of LC in the subsequent experiments. The results of our study showed that glucose consumption significantly decreased in LC-treated stored PC on day 5 of storage ($p<0.01$), as compared with untreated PC. Lactate concentration was lower in LC-treated stored PC on day 5 of storage as well ($p<0.05$). It seems that LC could reduce glycolysis and consequently reduce lactate accumulation resulting in better maintenance of pH of LC-treated stored PC. LC may increase the rate of fatty acid oxidation, permitting a reduction of glucose utilization. According to our results, bicarbonate concentration was better controlled in the LC-treated stored PC. In this regard, HCO_3 concentration showed more decline in the control group during storage days 2 to 5 ($p=0.017$). Holme et al. [18] and Dumont et al. [37] showed that bicarbonate depletion was closely related to a pH fall and an increase in lactate concentration in the PC. On the other hand, results of our study showed that LC-treated PC consumed more oxygen than that

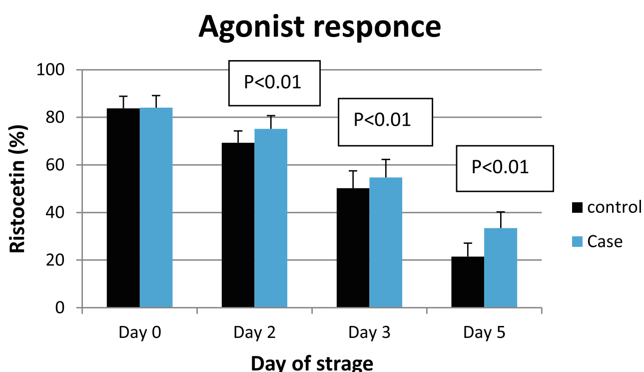

Fig. 2 Platelet aggregation in response to ristocetin at different days of storage in LC-treated PC (case) and PC without LC (control)

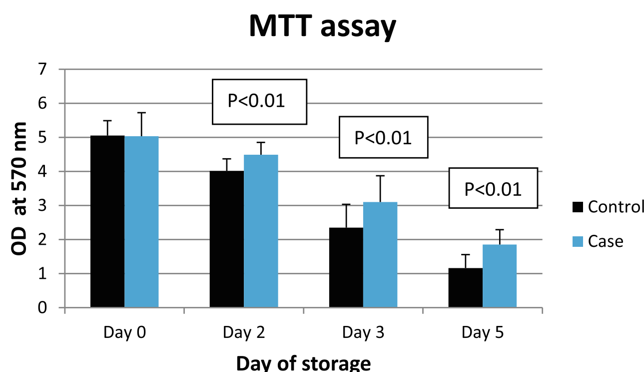

Fig. 4 MTT assays at different days of storage in LC-treated PC (case) and PC without LC (control)

untreated PC. In theory, increasing of fatty acid oxidation by LC indicated that platelet consumed oxygen more than that before. When PC consumes more oxygen, most of the ATP will be supplied by the aerobic oxidative phosphorylation leading to decreased ATP generation through glycolysis pathway, resulting in the decrease of lactate production and prevention of the fall in pH [26]. According to Tynngard's research [2], higher oxygen consumption in PC accompanied with decreased glycolysis and lactate accumulation results in increased platelet survival [2]. Our results are consistent with the previous studies presented by Sweeney et al. [26, 38]. They showed that LC at the concentration of 5 mM could be effective and useful contributor to improve platelet quality on the pH during storage of platelets [38]. In this study, LDH enzyme activity less significantly increased in LC-treated PC between day 3 and day 5 of storage ($p<0.05$) as compared with the control group. Due to integrity of cell membrane determined by the release of LDH, decrease in LDH release from the cytoplasm and supplement of enough oxygen for platelet metabolisms are both important factors for improving survival and quality of PC during storage [2]. It seems that LC-treated stored PC had less membrane damage than that untreated ones and LC could have beneficial effects on the PC quality. Regarding ATP levels, it was better maintained in LC-treated PC compared to the control group. But, the differences were not significant. It should be noted that the total intracellular ATP measured in this study and it was not possible to determine whether ATP levels between cytosolic and mitochondrial sources of ATP in two groups of PC were altered. However, decreasing glucose consumption and consequently reducing lactate accumulation were shown in LC-treated PC indicating that the glycolytic pathway is not the main source of energy in LC-treated PC. On the other hand, LC could help to recycle ATP by transporting fatty acids across the mitochondrial membranes [23], and it seems that the most of ATP in LC-treated PC derived by β-oxidation of long chain fatty acids in the platelet mitochondria. To assess the mitochondrial metabolic activity of stored PC, we used MTT method which is a sensitive assay for detection of normal metabolic status of cells. Platelets, like other cells, have mitochondria; thus, MTT method can evaluate the platelet mitochondrial enzyme activities related to platelet mitochondrial function, and it can be used to recognize the platelet metabolic status during storage [39–41]. In this regard, our result indicated that the mitochondrial respiration in LC-treated stored PC was better preserved than untreated PC on all days of storage ($p<0.01$). Mitochondrial metabolic activity was highly correlated with the oxidative pathway in platelets [41]. The oxidative pathway appeared impaired during platelet storage, but it seems that this pathway was improved in the presence of LC. Our data showed that LC could influence the platelet mitochondrial function through the protective effects on the mitochondrial respiration which is consistent with Ye J et al. [42]. They demonstrated that the supportive effect of LC on cell viability was related to its antioxidant property for protecting mitochondrial function. Overall, it seems that due to the increased oxidation of fatty acids in LC-treated PC, oxygen consumption would probably increase, and it might be an advantage of using LC for improving metabolic process of PC. LC could be a useful contributor to improve the quality of PC during storage. The other purpose of this study was to consider the platelet functionality in presence and absence of LC. So, according to previous data, we used the ristocetin and arachidonate as the agonist-induced platelet aggregation [43, 44]. We found that LC-treated stored PC had a significantly better aggregation response to the agonists. In this regard, responses to both of those agonists were higher in LC-treated stored PC than that untreated ones during all days of storage ($p=0.001$, $p=0.002$) which is consistent with Mincho et al. reports [45]. They indicated that LC-treated platelets potentially have a better response in platelet aggregation test. They also showed that LC could increase acethyl-CoA in platelet cytoplasm and it is necessary for excessive platelet aggregation. So, it can be concluded that LC could potentially increase platelet aggregation and it can cause an improvement in platelet function during storage. In case of MPV, it does indeed change during storage; it also considered representative of storage-induced shape changes in platelets [46, 47]. According to our results, MPV in LC-treated PC was better maintained ($p<0.01$) on day 5 of platelet storage, and less significant changes were observed in LC-treated PC compared to the control group during storage. It seems that LC could maintain the platelet shape during storage according to MPV index. Thus, LC may improve platelet quality during storage by this way. The advantage of our study is that we have used different doses of LC for treating PC. However, we showed that all concentrations of LC had beneficial effects on the PC, but the concentration of 15 mM was the best. As far as we know, this is the first report in this regard. The results of this study suggest that LC can be considered as an additive in maintaining the quality of stored PC and reduce PSL during storage. As we know well, finding the ways to reduce the PSL can help better performance of PCs for platelet therapy. It was noteworthy that LC was a naturally occurring substance and would not be expected to cause allergic or metabolic adverse effects.

Conclusion

LC at the concentration of 15 mM could be used as an additive for stored PC to improve the platelet metabolism, quality, and function during 5 days of storage. These data could be regarded preliminary steps for quality of PC in platelet transfusion therapy to be improved. Nevertheless, further in vitro and in vivo investigations need to be carried out to understand

the roles and effectiveness of LC in the metabolism and survival of PC.

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

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