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Antioxidative effects of α-tocopherol on stored human red blood cell units

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Abstract:

BACKGROUND: Red blood cell (RBC) units undergo metabolic, structural, and biochemical changes known as "storage lesions" that can reduce the survival and quality of RBCs. The use of antioxidants such as α-tocopherol may help to improve the quality of RBC units by reducing oxidative stress. The aim of this study was to determine the antioxidant effect of α-tocopherol in RBC units containing citrate-phosphate-dextrose solution with adenine (CPDA1) stored at 1°C–6°C for 35 days.

MATERIALS AND METHODS: Four RBC units containing CPDA1 were divided into four equal satellite bags. Three bags were supplemented with 0.125, 0.625, and 3.125 mM concentrations of α-tocopherol as test groups. One bag was supplemented with ethanol (0.5%) as a control group. They were stored at 1°C–6°C for 35 days. Malondialdehyde (MDA) concentration, total antioxidant capacity (TAC), and hemolysis index (HI) were measured on days 0, 7, 14, 21, 28, and 35.

RESULTS: In all groups, MDA concentration and HI increased and TAC decreased ($P < 0.05$). MDA concentration and HI in the 3.125 mM of the α-tocopherol group had a lower increase compared to the other test and control groups. Supplementation of RBC units with α-tocopherol resulted in a significant increase of TAC in all three groups compared to the control group ($P < 0.05$) and had a lower reduction during storage.

CONCLUSION: Supplementation of RBC units with α-tocopherol improves the quality of RBC units by decreasing lipid peroxidation and hemolysis and by increasing TAC. Among the mentioned concentrations, 3.125 mM of α-tocopherol had a significantly more antioxidant effect.

Keywords:

Hemolysis index, malondialdehyde, red blood cell unit, storage lesion, total antioxidant capacity, α-tocopherol

Introduction

Red blood cell (RBC) transfusion is widely used for patients with impaired oxygen delivery, including acute or chronic blood loss, increased RBC destruction, or deficiency in RBC production.[1,2] RBC unit due to the use of preparation methods, appropriate storage additive/preservative solutions (citrate-phosphate-dextrose [CPD]), saline-adenine-glucose-mannitol (SAGM) or Optisol (AS-5), poly Vinyl chloride blood bags, and storage at 1°C–6°C can be stored for up to 42 days.[3] Nevertheless, stored RBCs undergo metabolic, structural, and biochemical changes, which are collectively termed RBC storage lesions.[4-6] In summary, storage lesion includes acidosis, a decline of the endogenous antioxidant defense system, a decrease in the production of adenosine triphosphate, a decrease in the concentration of 2,3-diphosphoglycerate, leakage of hemoglobin (Hb), lactate, lactate dehydrogenase, and potassium ions into the supernatant, oxidative damage to the RBC membrane lipids and proteins, membrane microvesiculation, loss of RBC deformability, increased hemolysis, and finally cause a decrease in function and survival of transfused RBCs in the recipient.[7-9] One of the important factors

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in the RBC storage lesion is oxidative stress (OS), which occurs during the storage and due to the continuous contact of RBCs with oxygen, as a strong oxidant. OS occurs due to an imbalance in the production of free radicals and antioxidant capacity, which lead to the oxidation of proteins as well as the peroxidation of lipids in the membrane.[2,10] RBCs have two distinct antioxidant systems, enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and nonenzymatic (glutathione, Vitamins C, E, and A).[11]

Using preservatives enriched with antioxidants in RBC units can help to improve the quality and reduce storage lesions by reducing OS.[4] Vitamin E, which is part of the nonenzymatic antioxidant system, can overcome OS.[12] Alpha-tocopherol, the main form of Vitamin E, is a fat-soluble antioxidant in cell membranes, such as RBCs, and plays an important role in minimizing lipid peroxidation as a free radical scavenger and maintaining membrane stability. Its deficiency leads to an increase in membrane fragility and a decrease in cell survival under OS conditions.[12-17] Over the years, α-tocopherol has been considered a lipid-soluble antioxidant, and several studies have shown its activity on lipids by reducing the peroxidation of fatty acids of membranes.[4,12,18] This study aimed to evaluate the effect of different concentrations of α-tocopherol as a nonenzymatic antioxidant on hemolysis percentage, Malondialdehyde (MDA) concentration, and total antioxidant capacity (TAC) in RBC units containing CPD solution with adenine (CPDA-A1) stored at 1°C–6°C for 35 days.

Materials and Methods

Red blood cell units collection

This study has been approved by the Medical Ethics committee of the Iranian blood transfusion organization (IBTO) under (IR.TMI.REC.1400.002). RBC units with CPDA1 from 4 eligible, volunteer adult donors were obtained from the IBTO. A written informed consent form was obtained before blood donation. All units were screened for HIV, HCV, HBV, and syphilis infections and passed the release process under IBTO standard protocols.

Chemicals and kits

Alpha-tocopherol (T3251, DL-all-rac-α-tocopherol) was purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). MDA and TAC assay kits were purchased from Teb Pazhouhan Razi (TPR) (Tehran, Iran). The total Hb kit was obtained from Ziestchem Diagnostics (Tehran, Iran).

Alpha-tocopherol solution preparation

The alpha-tocopherol stock solution was prepared in ethanol to a concentration of 1000 mM. The final concentrations (0.125, 0.625, and 3.125 mM) of α-tocopherol in each bag were made by ethanol. The final ethanol concentration did not exceed 0.5% in each bag; therefore, for each RBC unit with a different volume, the amount of required α-tocopherol was calculated before use. Ethanol-control, phosphate-buffered saline (PBS) containing 0.5% final ethanol concentration was prepared for discrimination between the effects of α-tocopherol and ethanol on RBCs.

Sample preparation

Each RBC unit was equally divided into four satellite bags, of which three bags were treated with different concentrations of α-tocopherol (0.125, 0.625, and 3.125 mM) and one bag was treated with ethanol (0.5%, final ethanol concentration in bags) as a control. Subsequently, the units were stored at 1°C–6°C for 35 days under the standard conditions. Before grouping, an initial baseline sample was collected on the day of donation (day 0) aseptically.

Subsequent samples (5 ml) were collected weekly from each RBC unit by a sterile sampling device in a laminar airflow cabinet on days 7, 14, 21, 28, and 35 of storage after gentle mixing by inversion. Hb and hematocrit (HCT) were analyzed using XS-1000i Hematology-analyzer (Sysmex, Kobe, Japan). For lipid peroxidation (MDA measurement), TAC and hemolysis index (HI) samples were centrifuged at 3000 ×*g* for 10 min, and the supernatant was separated and stored at −20°C.

Lipid peroxidation measurement

Lipid peroxidation was assessed based on the measurement of MDA concentration, as an end product of lipid peroxidation, in supernatant plasma, using commercially MDA assay kit (Teb Pazhouhan Razi [TPR], Tehran, Iran) according to the manufacturer's instructions. The MDA present in the plasma reacts with thiobarbituric acid (TBA) in 90°C–100°C and produces the MDA-TBA compound, which can be easily quantified colorimetrically (530–540 nm). MDA standard curve was used to quantify the results. MDA concentration is presented as micromole/l.

Total antioxidant capacity measurement

TAC of plasma was measured using commercially TAC assay kit (Teb Pazhouhan Razi [(TPR], Tehran, Iran) according to the manufacturer's instructions. The basis of this method, which is a colorimetric reaction, is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidant compounds. This kit utilizes a peroxidase chromogenic substrate, which produces a water-soluble chromogen upon oxidation by ferryl myoglobin radicals. The green chromogen formation rate is inhibited by the presence of antioxidants in environments and is measurable

photometrically (412 ± 7 nm), based on the standard curve. TAC is expressed in micromole/l.

Hemolysis measurement

The HI was calculated by measuring the concentration of Hb released from the RBCs in supernatant plasma. The amount of released Hb was determined based on the cyanmethemoglobin method using Drabkin's reagent and determining the absorbance at 540 nm. The Hb concentration is expressed in g/dl. The HI was calculated by the following formula:

HI (%) = plasma Hb (g/dl) × (100–HCT)/total Hb (g/dl) × 100

Statistical analysis

The results are presented as mean ± standard deviation of four experiments. The significance of differences between the mean values of various concentrations of α-tocopherol versus the control group was analyzed using the nonparametric Wilcoxon test. The differences between different days of each group (compare intergroups) were analyzed using the Nonparametric Friedman test. $P < 0.05$ were considered significant. Statistical analysis was performed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). All presented figures were prepared using GraphPad Prism version 8.4.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Effect of α-tocopherol on lipid peroxidation and total antioxidant capacity

The MDA assay was performed to quantify lipid peroxidation in the plasma (supernatant) of RBC units. As shown in Figure 1a, a time-dependent increase of the MDA level was observed in all groups ($P < 0.001$). Alpha-tocopherol supplementation of RBC units in high concentration (3.125 mM) significantly decreased MDA production during storage compared to the control group and other α-tocopherol-treated groups ($P < 0.05$) [Figure 1b]. However, there was no significant difference in MDA level between RBC stored with 0.125 and 0.625 mM of α-tocopherol and the control group ($P > 0.05$).

As shown in Figure 2a, the TAC level significantly decreased in a time-dependent manner ($P < 0.01$). Supplementation of RBC units with α-tocopherol resulted in a significant increase of TAC in all three test groups compared to the control group [$P < 0.05$ Figure 2b]. RBC TAC level was significantly higher on

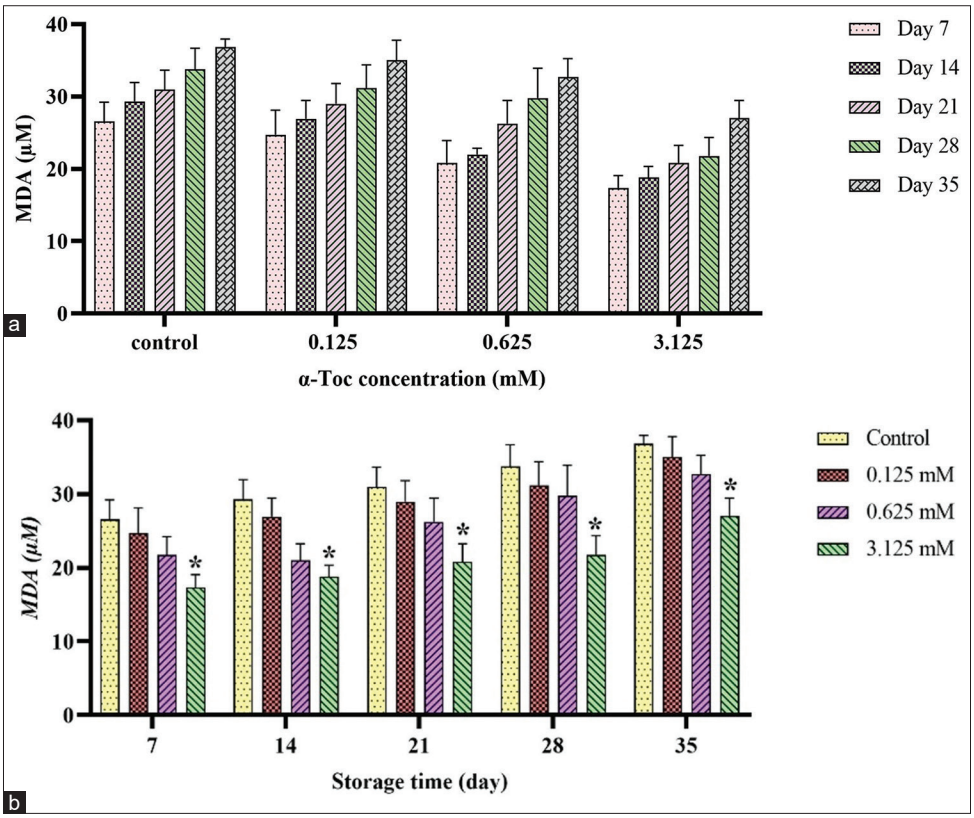

Figure 1: Effect of α-tocopherol on MDA concentration of the RBC units. The results are expressed as mean ± SD for 4 independent experiments. (a) The differences between different days of each group (compare intergroups) were analyzed by the Nonparametric Friedman test. $P < 0.001$ in each group. (b) The differences between the groups were analyzed using the nonparametric Wilcoxon test. *$P < 0.05$ in the presence of 3.125 mM of α-tocopherol compared to the control group. MDA: Malondialdehyde, RBC: Red blood cell, SD: Standard deviation

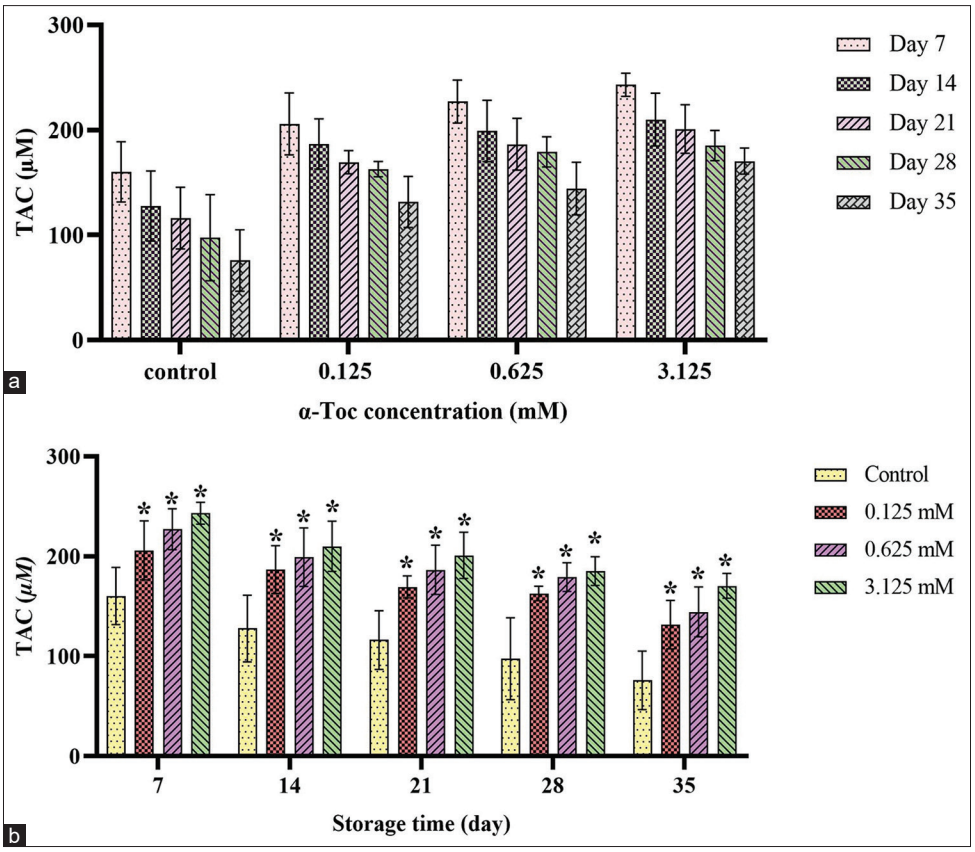

Figure 2: Effect of α-tocopherol on the TAC level of the RBC units. The results are expressed as mean ± SD for four independent experiments. (a) The differences between different days of each group (compare intergroups) were analyzed using the Nonparametric Friedman test. $P < 0.01$ in each group. (b) The differences between the groups were analyzed using the nonparametric Wilcoxon test. *$P < 0.05$ in the presence of different concentrations of α-tocopherol compared to the control group. TAC: Total antioxidant capacity, RBC: Red blood cell

the 7th day compared to the baseline, persisting until the last day of storage due to the addition of α-tocopherol as an antioxidant. Increased TAC by α-tocopherol showed the ability of that, particularly at 3.125 mM concentration, to restore and increase the TAC.

Effect of α-tocopherol on hemolysis

As shown in Figure 3a, hemolysis significantly increased during storage time ($P < 0.001$). In RBC stored with 0.125 and 0.625 mM of α-tocopherol, there were no significant differences of HI in comparison with the control group [$P > 0.05$, Figure 3b]. However, the HI of RBC units with 3.125 mM of α-tocopherol was significantly lower than that of the control group and other α-tocopherol concentrations ($P < 0.05$).

Discussion

Numerous studies have shown that increased storage lesions of the RBC units could result from a decrease in the antioxidant defense system, which increases the susceptibility of stored RBCs to OS.[19,20] In the present study, we assumed that supplementation of the RBC units with α-tocopherol could restore the antioxidant capacity and subsequently increase the antioxidant capacity of RBC against OS. OS results in lipid peroxidation and an increase in MDA. MDA is an end product of lipid peroxidation and an OS indicator. It cross-links the phospholipids and proteins of the erythrocyte membrane and impairs its functions, and finally decreases RBC survival.[19,21,22] Aslan *et al*.[23] demonstrated that plasma MDA levels of stored RBC increased with time.[23] In the current study, we also found that plasma MDA levels significantly increased during the storage in the RBC units. We evaluated the anti-oxidative effect of α-tocopherol against OS in RBC units and demonstrated that α-tocopherol, in a dose-dependent manner, decreased MDA levels in α-tocopherol-treated groups compared to the control group. Different concentrations of α-tocopherol had different effects on MDA levels. At a concentration of 3.125 mM, α-tocopherol was more effective than other concentrations in decreasing MDA levels. The MDA level decrease in the α-tocopherol-treated group compared to the control group can be related to the effect of α-tocopherol to decrease lipid peroxidation, which is corroborated with the studies of Chung and Benzie,[24] and Racek *et al*.[25] This protective effect of α-tocopherol can be due to its hydrophobic nature. It can interact/penetrate in the membrane, thus stabilizing erythrocyte membrane components.[4]

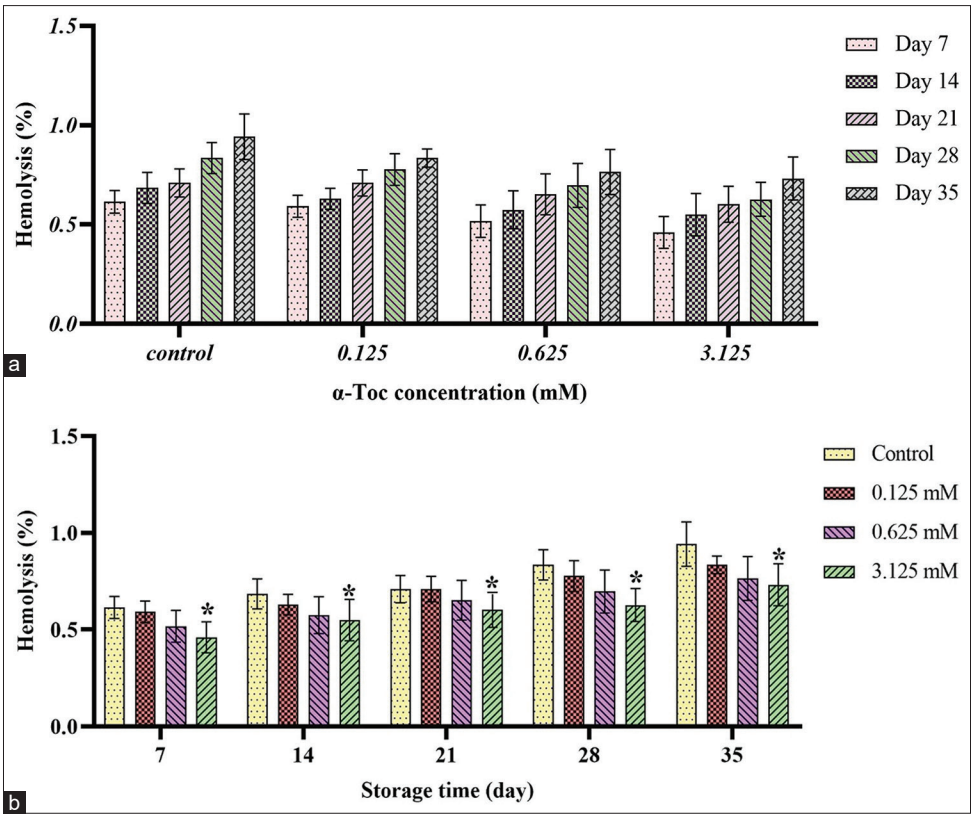

Figure 3: Effect of α-tocopherol on hemolysis of the RBC units. The results are expressed as mean ± SD for four independent experiments. (a) The differences between different days of each group (compare intergroups) were analyzed using nonparametric Friedman test. $P < 0.001$ in each group. (b) The differences between the groups were analyzed using the nonparametric Wilcoxon test. *$P < 0.05$ in the presence of 3.125 mM of α-tocopherol compared to the control group. RBC: Red blood cell, SD: Standard deviation

TAC was significantly decreased in RBC units in a time-dependent manner due to the oxidation of endogenous antioxidants ($P < 0.01$). However, it was significantly higher in all three test groups compared to the control group ($P < 0.05$). Indicating that α-tocopherol, as an exogenous antioxidant, protected the RBCs against OS by scavenging free radicals and restoring the TAC ability. Our results were in agreement with the results of Antosik *et al*.[4] study. They showed that RBC units stored in SAGM supplemented with Trolox (Vitamin E water-soluble analog) had significantly lower lipid peroxidation levels and higher TAC, compared to the RBCs stored in SAGM alone. The differences between these two studies were that they used RBC units containing SAGM, an additive solution.

The hemolysis and free Hb concentration of RBC units were also increased with time. Our results indicated that α-tocopherol, in a dose-dependent manner, decreases storage-induced hemolysis and free Hb concentration. RBC units with 0.125 and 0.625 mM concentrations of α-tocopherol did not have significant differences in hemolysis compared to the control group, although supplementation of RBC units with 3.125 mM of α-tocopherol led to lower hemolysis compared to the control group. Similarly, Sun *et al*.[26] demonstrated that Vitamin E supplementation significantly decreased erythrocyte hemolysis and improved membrane fluidity in healthy middle-aged and elderly people.

Conclusion

Our results demonstrated that in the RBC unit, hemolysis and lipid peroxidation increased and TAC decreased time dependently. However, supplementation of RBC units with α-tocopherol at the onset of storage improves the quality of RBC units by decreasing lipid peroxidation and hemolysis and by increasing TAC. These effects are concentration dependent and the high concentration of α-tocopherol (3.125 mM) had a significantly more antioxidative effect. Our study showed that a 3.125 mM concentration of α-tocopherol can be safely used in an RBC storage solution.

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Conflicts of interest

There are no conflicts of interest.

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