Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2018, Vol. 46(1) 272–283

DOI: 10.1177/0300060517725450
journals.sagepub.com/home/imr
SAGE

Characteristics of resveratrol and serotonin on antioxidant capacity and susceptibility to oxidation of red blood cells in stored human blood in a time-dependent manner

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Abstract

Objective: In stored red blood cells (RBCs), which are used in diseases (e.g., acute blood loss and leukaemia), storage lesions arise by oxidative stress and other factors over time. This study investigated the protective effects of resveratrol and serotonin on stored RBCs.

Methods: Blood from each donor (n = 10) was placed in different bags containing 70 mL of citrate phosphate dextrose (total volume: 500 mL) and divided into three groups (n = 30): control, 60 μg/mL resveratrol, and 60 μg/mL serotonin. Malondialdehyde (MDA) and glutathione (GSH) levels, activity of glutathione peroxidase (GSH-Px), catalase, and carbonic anhydrase (CA), and susceptibility to oxidation in RBCs, and pH in whole blood were measured at baseline and on days 7, 14, 21, and 28.

Results: MDA levels and susceptibility to oxidation were increased in all three groups time-dependently, but this increase was greater in the serotonin group than in the other groups. Activity of GSH-Px, CAT, and CA, as well as GSH levels, were decreased in the control and serotonin groups time-dependently, but were significantly preserved in the resveratrol group. The pH was decreased in all groups time-dependently.

Conclusion: Our study shows that resveratrol attenuates susceptibility to oxidation of RBCs and protects their antioxidant capacity, and partially preserves CA activity time-dependently.

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Keywords
Antioxidants, resveratrol, serotonin, storage lesions, stored RBCs, oxidation, chronic storage

Date received: 16 May 2017; accepted: 17 July 2017

Introduction

Red blood cells (RBCs), which are used for transfusion, can be stored for up to 6 weeks at 2–6°C under suitable conditions.[1] However, morphological, biochemical, and functional changes known as "storage lesions" can arise in RBCs at the initial stage of storage.[2] Storage lesions negatively affect the lifespan and efficiency of RBCs in a time-dependent manner.[3] The maximum storage period and effectiveness of RBCs depend on the content of their storage medium and protective solutions.[4] Various preservative solutions have been developed for minimizing storage lesions and prolonging the lifespan of RBCs.[5] The most common combinations involve addition of citrate phosphate dextrose (CPD), acid citrate dextrose, citrate phosphate dextrose adenine, and sorbitol glucose adenine mannitol.[6–8] Although blood can be stored in these solutions, storage lesions may develop.[3,5] Therefore, intensive studies have aimed to increase the storage times of RBCs. These studies reported that the addition of various antioxidants, such as vitamin E, vitamin C, β-carotene, melatonin, propofol, and tannic and caffeic acids, as protective solutions had a positive effect on the survival of RBCs.[5,9,10]

We hypothesize that the antioxidant properties of resveratrol and serotonin (phenolic compounds) [11,12] may benefit the susceptibility to oxidation and antioxidant capacity of RBCs in stored blood. This is because reactive oxygen species (ROS), such as hydroxyl radical (OH^-), superoxide anion radical ($^1O_2^-$), and hydrogen peroxide (H_2O_2), increase oxidative stress in a time-dependent manner.[10,13] ROS induce oxidation in membrane lipids and proteins, and antioxidants in the phenolic structure can scavenge the factors that cause oxidative stress.[14,15] Therefore, the lifespan of stored RBCs can be extended by reducing oxidative stress.

Resveratrol is a natural compound that acts on various intracellular messengers[11] and is found in edible plant leaves and red wine, as well as in fruits, such as mulberry, grapes, and peanuts.[16,17] Because of the beneficial effects of resveratrol on humans, resveratrol has become the focus of attention worldwide. Gülçin reported that resveratrol inhibited peroxidation of a linoleic acid emulsion by 89.1% and was more effective than standard antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, α-tocopherol, and Trolox.[16] This author also demonstrated that resveratrol was effective for its 1,1-diphenyl 2-picrylhydrazyl (DPPH), 2,2'-Azino-bis [3-ethyl benzo-thiazoline-6-sulfonic acid] ($ABTS^+$), N, N-dimethyl-p-phenylenediamine radical cation ($DMPD^+$), and O_2^- scavenging activity, H_2O_2 reduction power, and Fe^{2+}chelating activity. Gülçin also reported that resveratrol exhibited beneficial effects on cardiovascular diseases by promoting vasodilatation and inhibiting oxidative damage and platelet aggregation.[16]

Serotonin (5-hydroxytryptamine) is synthesized in Drosophila by tryptophan-phenylalanine hydroxylase activation, which hydroxylates phenylalanine and tryptophan.[18] Serotonin is an important neurotransmitter that has several biological roles.[19] $ABTS^+$ radical scavenging activity and antioxidant properties of serotonin and some of its derivatives have been tested. Gülçin found that melatonin and serotonin exhibited effective $DMPD^+$ radical

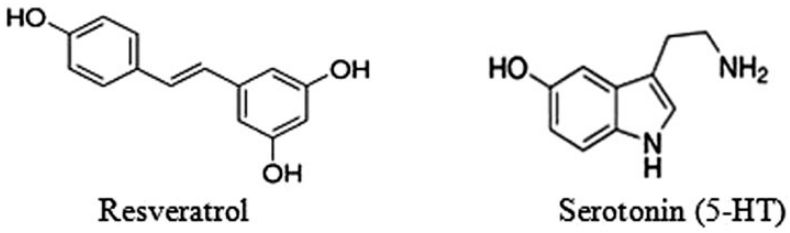

Figure 1. Apparent molecular structure of resveratrol and serotonin

scavenging and cupric ion (Cu^{2+}) reduction activity.[12] The molecular structures of resveratrol and serotonin are shown in Figure 1.

In this study, we investigated the protective effects of resveratrol and serotonin on lipid peroxidation and sensitivity to oxidation of RBCs in stored blood. We aimed to develop protective solutions and increase the viability of RBCs. The effects of resveratrol and serotonin on total carbonic anhydrase (CA), catalase (CAT), and glutathione peroxidase (GSH-Px) activity, as well as reduced glutathione (GSH) levels and pH, were also investigated.

Materials and methods

This study protocol was performed in accordance with the ethical rules in human experiments in the context of the World Medical Association Ethics Rules in the Helsinki Declaration. For this study, ethics committee approval was obtained from the Yuzuncu Yıl University Non-Pharmaceutical Clinical Practices Local Ethics Committee (REC number: 06/13.08.2014). Before blood was taken, necessary information was provided to the volunteers of the study.

Resveratrol and serotonin were obtained from Sigma Aldrich Company (St Louis, MO, USA). Four-way paediatric bag systems connected to the main bag for blood samples were purchased from Kansuk (LOT:1104070; Kansuk, Istanbul, Turkey). NADPH, molybdic acid, GSH, 1,1,3,3-tetraethoxy propane, methanol, ethanol, hydrogen peroxide, and other chemicals were obtained from Merck (Istanbul, Turkey).

Obtaining blood samples from volunteers and preparing for measurements of parameters

Blood from each healthy volunteer (n = 10) was collected in four different paediatric bag systems (Kansuk) containing 70 mL of CPD at the Yuzuncu Yıl University Medical Faculty Blood Center. Blood was transferred to three paediatric bags attached to the main bag and categorized into three groups (n = 30). One group was the control group; 60 μg/mL resveratrol (resveratrol group) or 60 μg/mL serotonin (serotonin group) was added to blood for the other groups. Blood was stored at +4°C in a blood bank during the study.

Blood samples (12 mL, n = 30) were taken from stored blood in each group at 1-week intervals (weeks 0, 1, 2, 3, and 4). Measurement of pH was performed using 2 mL of whole blood. The remaining 10 mL of whole blood samples were centrifuged at 2500 × g for 15 min and the plasma was discarded. Upper leucocytes and other impurities were carefully removed using a dropper. An equal volume of isotonic solution was added to the underlying erythrocyte (RBC) solution and was slowly overturned. The supernatant was discarded

after centrifugation at 2500 × g. This procedure was repeated three times and thorough washing of the RBCs was achieved. The susceptibility to oxidation of RBCs was measured in an erythrocyte suspension that was prepared in azide buffer solution. The remaining RBC packages were haemolysed with distilled ice water and frozen at −80°C until measurement of GSH levels, and GSH-Px, CAT, and CA activity.

Haemoglobin measurement in RBC packages

Haemolysate (20 µL) was added to a 5-mL drabkin solution and incubated at room temperature for 10 min. The absorbance of the mixture was measured colourimetrically on a spectrophotometer (Shimadzu UV mini 1240; Korea, Japan) at 540 nm. Haemoglobin concentrations are expressed as g/dL.[20]

Measurement of MDA by high-performance liquid chromatography (HPLC)

Briefly, 250 µL thiobarbituric acid (42 mM), 750 µL H_3PO_4 (0.44 M), and 450 µL distilled water were added to the 50-µL RBC suspension in a test tube and the mixture was incubated for 60 min in a boiling water bath (total volume: 1.5 mL). After cooling in ice water, the same volume of alkaline methanol (50 mL methanol + 4.5 mL of 1 M NaOH) (1/1 v/v) was added to the test tube and centrifuged at 3000 × g for 3 min. The supernatant (200 µL) was transferred to a vial and injected into an HPLC apparatus (Agilent, Boblingen, Germany). An RP-18 column (150 ×4.6 mm) and 5-μm particle size were used. The mobile phase consisted of a mixture of 400 mL of 50-mM phosphate buffer (pH 6.8) and 600 mL of methanol (total volume 1 L). The flow rate of the mobile phase and the injection volume of samples were set to 0.8 mL/min and 20 µL, respectively. Measurements were made against standard samples of different concentrations at an excitation wavelength of 527 nm and an emission wavelength of 551 nm.[21] MDA levels are expressed as nmol/gHb.

Determination of susceptibility to oxidation of RBCs

The susceptibility to oxidation of RBCs *in vitro* was determined using the method of Stocks et al.[21] The RBC suspension was prepared with azide buffer. A volume of 5 mL of H_2O_2 (0.03% concentration prepared in buffered saline solution) was then added to the same RBC suspension, and the mixture was incubated for 2 h at 37°C for oxidation. After 2 h, MDA levels were measured on the HPLC apparatus,[22] and the haemoglobin concentration of the RBC suspension was determined. MDA values are expressed as nmol/gHb.

Measurement of GSH-Px activity in RBCs

Erythrocyte GSH-Px activity was determined using the method described by Paglia and Valentin.[23] In this method, GSH reductase transforms oxidized GSH to reduced GSH in the presence of NADPH, accompanying the conversion of NADPH to $NADP^+$. For this purpose, a 970-µL reaction mixture (mixture of Tris, Na_2EDTA, GSH, NADPH, sodium azide, and GSH reductase) was added to 20 µL of sample and incubated for 5 min at 37°C. After incubation, 10 µL of H_2O_2 solution was added to initiate the reaction, and decreasing absorbance values were measured at 340 nm at 30-s intervals for 3 min. GSH-Px activity is expressed as IU/gHb.

Measurement of CAT activity in RBCs

CAT activity in RBCs was measured according to the colourimetric method of Goth.[24] A volume of 1 mL of 65 μmol/mL

H_2O_2 (prepared in phosphate buffer 0.06 M, pH of 7) was added to the 0.2-mL RBC package. After 1 min, 1 mL of ammonium molybdate solution (32.4 mmol/L) was added to the test tube to terminate the reaction. Absorbance of the yellow complex formed by molybdate and H_2O_2 was measured spectrophotometrically at 405 nm on a spectrophotometer (Shimadzu UV mini 1240). CAT activity is reported as kU/gHb.

Measurement of reduced GSH in RBCs

GSH values in RBCs were measured spectrophotometrically according to the method of Fairbanks and Klee.[25] The non-protein sulfhydryl groups in RBCs are normally in the form of reduced GSH. DTNB, a disulphide chromogen, is reduced to a yellow compound by the sulfhydryl groups of the GSH in RBCs. Absorbance of the reduced chromogen shows maximum absorbance at 412 nm. The concentration of this complex is directly proportional to the GSH concentration. Although the DTNB method measures all free sulfhydryl groups (protein-bound or protein unbound), the sulfhydryl groups are thought to reflect the GSH value in RBCs because GSH levels are high in RBCs. GSH values are expressed as mg/gHb.

Determination of total CA activity in RBCs

Total CA activity in RBCs was measured using the method described Rickly et al.[26] and modified by Wilbur and Anderson.[27] Purified water (0.55 mL), bromothymol blue (0.1 mL, 0.04%), and haemolysate (50 µL enzyme solution) were added to tubes containing 1 mL of veronal buffer (0.025 M, pH of 8.2). The elapsed time for the conversion from the blue colour of the indicator to the yellowish-green (pH 6.8) colour was determined by addition of 2.5 mL of CO_2 solution (total volume: 4.2 mL) as the substrate (t_c). As a reference measurement, we used the elapsed time for the colour change using distilled water instead of haemolysate (t_0). CA activity (CO_2−hydratase activity) was calculated using the $(t_0 - t_c)/t_c$ equation[28,29] and is expressed as U/gHb.

Measurement of pH

Values of pH were measured with a commercially purchased blood gas kit (ABL90 FLEX PLUS, Radiometer, Denmark) on an ABL 90 apparatus (Radiometer Medical Materials Industry and Trade Ltd. Inc., Istanbul, Turkey).

Statistical analyses

Descriptive statistics for continuous variables are expressed as the mean and standard deviation. Following analysis of variance, Duncan's multiple comparison test was used for inter-group comparison within the same week and for intra-group comparison between weeks. Statistical significance was considered at 5% ($p < 0.05$). The SPSS 15 statistical package program (Chicago, IL, USA) was used for calculations.

Results

Volunteers involved in the study ($n = 10$) were people who had lived in Van city centre in Turkey for at least 2 years. Four of the volunteers were Kurdish and six of them were of Turkish origin. Volunteers were healthy men between the ages of 21 and 25.

MDA levels in RBCs in all of the groups increased in a time-dependent manner ($p < 0.05$). However, this increase was greater in the serotonin group from week 3 onwards (on days 14, 21, and 28) compared with the other groups ($p < 0.05$) (Figure 2). We also tested the susceptibility of RBCs to oxidation *in vitro*. Two hours after *in vitro* oxidation with H_2O_2 (0.03%),

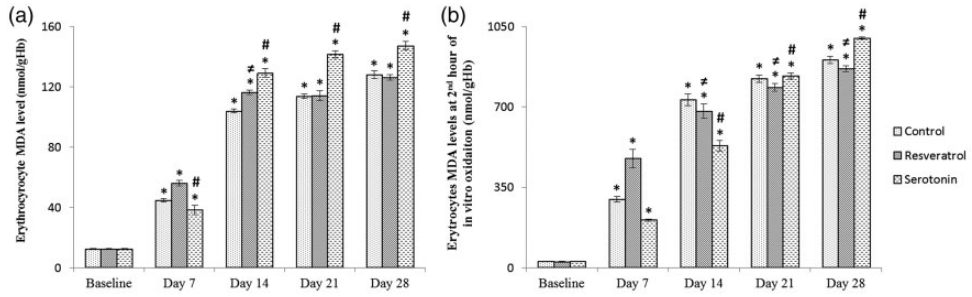

Figure 2. Effect of resveratrol and serotonin on malondialdehyde levels in red blood cells
A: Malondialdehyde values at the beginning of *in vitro* oxidation with 0.03% H_2O_2. B: Malondialdehyde values after 2 h of *in vitro* oxidation with 0.03% H_2O_2. $^{*}p < 0.01$ versus baseline; $^{\neq}p < 0.001$ versus controls; $^{\#}p < 0.001$ versus resveratrol and controls.

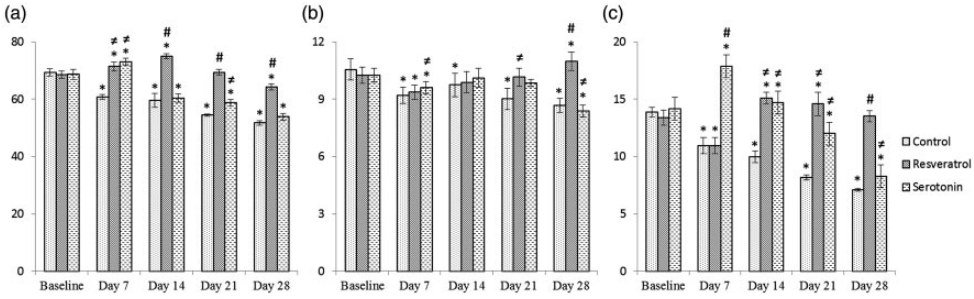

Figure 3. Protective effects of resveratrol and serotonin on glutathione peroxidase and catalase activity, and glutathione levels in red blood cells
A: glutathione peroxidase activity (U/gHb); B: glutathione levels (mg/gHb); and C: catalase activity (kU/gHb). $^{*}p < 0.01$ versus baseline; $^{\neq}p < 0.001$ versus controls; $^{\#}p < 0.001$ versus resveratrol and controls.

MDA levels were increased in all groups in a time-dependent manner during 5 weeks. However, after *in vitro* oxidation, MDA levels in the resveratrol group were significantly lower than those in the other groups on days 14, 21, and 28 ($p < 0.05$). After *in vitro* oxidation with H_2O_2, MDA values on days 21 and 28 in the serotonin group were significantly higher than those in the control and resveratrol groups ($p < 0.05$) (Figure 2).

We also compared changes in GSH-Px and CAT activity and levels of GSH, which function as antioxidants in RBCs, over time. GSH-Px and CAT activity, as well as GSH levels, were decreased in the control and serotonin groups, but were significantly preserved in the resveratrol group in a time-dependent manner ($p < 0.001$). GSH-Px and CAT activity, as well as GSH levels, in the resveratrol group were also significantly higher than those in the other groups on days 21 and 28 ($p < 0.001$) (Figure 3). However, GSH-Px and CAT activity, as well as GSH levels, did not significantly change in the serotonin group, and were similar to those of the control group.

We further examined the change in CA activity, which provides pH and ionic balance in RBCs over time. CA enzyme activity in all of the groups was decreased in

intra-group comparisons in a time-dependent manner ($p < 0.001$). However, CA activity of the resveratrol group was significantly higher than that in the other groups on days 21 and 28 ($p < 0.05$) (Figure 4).

We investigated changes in pH and pCO_2 in stored whole blood in association with CA time-dependently. We found that pH was decreased in all groups time-dependently ($p < 0.05$). However, pH values on day 28 in the resveratrol group were partially preserved according to the control group, although this was not significant (Figure 4). Additionally, pCO_2 values rapidly increased during the first 3 weeks, and then began to fall ($p < 0.05$). However, pCO_2 values in the resveratrol group were lower than those in the other groups on day 28 ($p < 0.05$) (Figure 4).

Discussion

We investigated the effects of resveratrol and serotonin on antioxidant defence status and oxidative alterations that distort the physiological functions of RBCs. We also examined their protective effects on CA enzyme activity, which plays important roles, such as pH and ion balance in the body.

Storage lesions are defined as changes that result in irreversible damage and decrease post-transfusion survival of blood or RBCs.[30] The most obvious changes that affect RBCs during storage are an increase in lipid peroxidation levels and a decrease in the activity of enzymes that play a role in the antioxidant defence system, such as superoxide dismutase (SOD), CAT, and GSH-Px. Additionally, there are haemorheological changes, such as an increase in RBC sphere membrane deformation and mean Hb concentrations, and osmotic fragility.[30,31]

Most irreversible events occur as a result of long-term oxidative stress under non-physiological storage conditions. One of the most important reasons for an increase in oxidative stress is decreased antioxidant capacity of RBCs or stored blood.[32] ROS, which result from increasing oxidative stress in stored RBCs, lead to impaired membrane integrity and cell death by attacking lipids and proteins in membranes.[30] Therefore, cells possess enzymatic or non-enzymatic antioxidant defence mechanisms against this situation.[31,32] However, researchers have suggested that these negative changes occur even if stored RBCs or blood are maintained under suitable conditions. Various studies have been

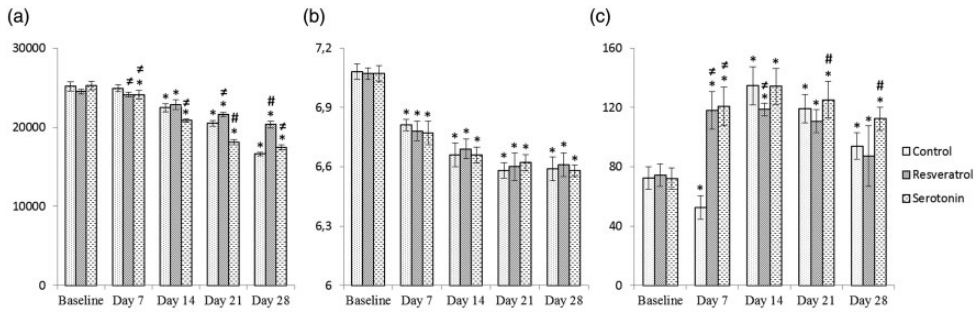

Figure 4. Protective effects of resveratrol and serotonin on total carbonic anhydrase activity in red blood cells, as well as pH and pCO_2 in whole blood
A: Carbonic anhydrase activity (U/gHb); B: pH; and C: pCO_2. *$p < 0.01$ versus baseline; $^{\neq}p < 0.001$ versus controls; $^{\#}p < 0.001$ versus resveratrol and controls.

performed on stored blood to decrease the observed negative changes in a time-dependent manner and to increase the life-span of RBCs.[5,6,32,33]

Dumaswala et al.[34] showed that an increasing concentration of GSH in stored blood protected RBCs against free radical damage. Additionally, numerous studies have shown that lipid peroxidation is attenuated by the addition of metal chelators and antioxidant substances to stored blood or RBCs.[32,35,36] Before blood is taken, donors are provided various antioxidant preparations in their diet. Racek et al.[37] showed that stored blood was more resistant to free radical damage if various antioxidant tablets were provided to volunteers before blood collection.

Melatonin is one of the strongest antioxidants.[38] Allegra et al.[38] demonstrated that erythrocyte membrane proteins and lipids increased the susceptibility to oxidation with a cytotoxic effect of MDA *in vitro*. However, addition of melatonin to stored blood resulted in a protective effect of RBCs against these adverse events. Sekeroglu et al.[5] found that addition of melatonin to stored blood significantly preserved the levels of erythrocyte MDA, glutathione, GSH-Px, and SOD in a time-dependent manner. In previous studies, MDA levels in erythrocytes increased in a time-dependent manner.[5,9,39] Our study showed that MDA levels increased in RBCs in a time-dependent manner, consistent with these previous studies. We also tested the susceptibility of RBCs to oxidation *in vitro*. The susceptibility to oxidation of RBCs was increased under conditions of oxidative stress by addition of H_2O_2 over time. This result supports the hypothesis that some changes occur in RBC membranes because of oxidative damage during the waiting period and that RBCs become less stable. In our study, resveratrol added to stored blood significantly preserved the susceptibility to oxidation of RBCs compared with the control group. However, serotonin showed an adverse effect.

In addition to the endogenous antioxidant systems that play a protective role against oxidative stress in RBCs, there are other factors, such as SOD, GSH-Px, CAT and GSH.[30,31] GSH-Px acts as the primary antioxidant defence system and performs decomposition of H_2O_2 at high H_2O_2 concentrations.[40] CAT completes the detoxification reaction of H_2O_2 initiated by SOD to H_2O and O_2.[41] CAT activity is initially low and increases with storage time.[5,32] This situation may be due to activity of GSH-Px that scavenges H_2O_2 at low H_2O_2 concentrations. Previous studies have shown that an increasing storage time decreases GSH-Px activity.[5,31] Our study suggested that GSH-Px activity in RBCs was attenuated with increasing storage time, in accordance with previous studies.[5,31] However, addition of resveratrol and serotonin to stored blood increased GSH-Px activity in RBCs during the first 2 weeks and significantly protected RBC in the following weeks compared with the control group.

CAT and NADPH protect RBCs against acute and high exogenous H_2O_2 levels, while GSH protects RBCs against endogenously produced low H_2O_2 levels.[42] In a previous study conducted by our group, CAT activity and GSH levels increased during the first 2 weeks and decreased in the following weeks.[5,32] Our current study showed that CAT levels in the resveratrol and serotonin groups increased during the first 3 weeks and were then attenuated in the following weeks, consistent with previous studies.[5,31,32] However, when we examined total CAT levels at the end of day 28, CAT levels in the control and serotonin groups were significantly lower compared with the resveratrol group. Additionally, similar changes in GSH and CAT levels showed a significant association between

these two parameters. The higher levels of CAT and GSH in the resveratrol group on days 21and 28 may be due to the antioxidant properties of resveratrol.

CAs (carbonate hydrolase, EC 4.2.1.1) represent a large family of Zn-containing enzymes.[43] A total of 16 different CA isoenzymes have been identified in mammals.[44,45] In various tissues, different CA isoenzymes participate in many important biological processes, such as respiration, gas balance, calcification, photosynthesis, bone resorption, lipid synthesis, urea synthesis, glucose synthesis, body fluid exchange, tumour cell growth, electrolyte release, CO_2 and ion transport, and acid-base balance by regulating pH in the eye and inner ear.[46,47] CAs contribute to pH balance in the human body and other tissues by catalysing two steps: providing H^+ and HCO_3^- with hydration of CO_2 and converting HCO_3^- to CO_2 in acidic medium.[48] CA activity is important because a drop in pH has a negative effect on stored RBCs.[32] There have been few studies on stored blood or RBCs concerning how CA activity changes over time. In a previous study carried out by our group, CA activity decreased in a time-dependent manner.[32] In the current study, CA activity was attenuated in a time-dependent manner, similar to our previous study.[32] The decrease in CA enzyme activity over time may be due to a loss of function of proteins depending on the elapsed time, as in other enzymes. However, resveratrol may protect stored blood and RBCs against time-related acidosis, as indicated by the partially preserved CA activity, and pH and pCO_2 values on days 21 and 28 in the resveratrol group. In our previous study, resveratrol (30 μg/mL) significantly preserved the change in pH in stored RBCs.[29] We believe that the reason why resveratrol did not exhibit a protective effect on the change in pH in this study may be because resveratrol was used at a higher dose (60 μg/mL).

Conclusions

Our study shows that the antioxidant capacity of RBCs is attenuated because of their increasing susceptibility to oxidation and decrease in GSH levels, and GSH-Px and CAT activity, in a time-dependent manner. Furthermore, the decrease in GSH-Px and CAT activity and GSH levels in RBCs is negatively associated with CA enzyme activity and antioxidant capacity with susceptibility to oxidation over time. However, addition of resveratrol to stored blood protects their antioxidant capacity and partially preserves CA enzyme activity and pH and pCO_2 levels by decreasing the susceptibility to oxidation of RBCs. For this reason, low concentrations of resveratrol that are added to stored blood may positively contribute to the shelf life of stored RBCs. However, the dose of resveratrol that should be added to stored blood or RBCs must be adjusted accordingly. More detailed studies are required to determine the mechanism by which serotonin increases the susceptibility to oxidation.

Declaration of conflicting interest

The Authors declare that there is no conflict of interest.

Funding

This study was supported by the Department of Biochemistry, Yuzuncu Yıl University, Faculty of Medicine.

References

1. Aleshnick M, Foley JH, Keating FK, et al. Procoagulant activity in stored units of red blood cells. *Biochem Biophys Res Commun* 2016; 474: 680–685.
2. Desmarets M, Bardiaux L, Benzenine E, et al. Effect of storage time and donor sex of transfused red blood cells on 1-year survival in patients undergoing cardiac

surgery: an observational study. *Transfusion* 2016; 56: 1213–1222.
3. Park H, Lee S, Ji M, et al. Measuring cell surface area and deformability of individual human red blood cells over blood storage using quantitative phase imaging. *Sci Rep* 2016; 6: 34257 doi:10.1038/srep34257.
4. Beutler E. Preservation and clinical use of erythrocytes and whole blood. In: Marshall AL, Ernest B, Thomas JK, Uri S, Kenneth K, Josef TP (eds). Williams hematology The Mc Graw-Hill Companies 2006, pp. 2159–2163.
5. Sekeroglu MR, Huyut Z and Him A. The susceptibility of erythrocytes to oxidation during storage of blood: Effects of Melatonin and Propofol. *Clin Biochem* 2012; 45: 315–319.
6. Mustafa I, Al Marwani A, Mamdouh Nasr K, et al. Time dependent assessment of morphological changes: Leukodepleted packed red blood cells stored in SAGM. *BioMed Res Int* 2016; 2016: 4529434, DOI:10.1155/2016/4529434, 7 pages.
7. Valeri CR, Valeri DA, Gray A, et al. Viability and function of red blood cell concentrates stored at 4 °C for 35 days in CPDA-1, CPDA-2, or CPDA-3. *Transfusion* 1982; 22: 210–216.
8. Van't Erve TJ, Wagner BA, Martin SM, et al. The heritability of hemolysis in stored human red blood cells. *Transfusion* 2015; 55: 1178–1185.
9. Racek J, Herynkova R, Holecek V, et al. Influence of antioxidants on the quality of stored blood. *Vox Sang* 1997; 72: 16–19.
10. Huyut Z, Şekeroğlu MR, Balaharoğlu R, et al. In stored human blood, the inhibitor effect of tannic acid and caffeic acid on lipid peroxidation and oxidative DNA damage. *Eastern J Med* 2016; 21: 88–93.
11. Borges SC, da Silva de Souza AC, Beraldi EJ, et al. Resveratrol promotes myenteric neuroprotection in the ileum of rats after ischemia-reperfusion injury. *Life Sci* 2016; 166: 54–59.
12. Gülçin I. Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent. *J Enzyme Inhib and Med Chem* 2008; 23: 871–876.
13. Ravikumar S, Hsieh C and Rajashekharaiah V. Prospects of curcumin as an additive in storage solutions: a study on erythrocytes. *Turk J Med Sci* 2016; 46: 825–833.
14. Mohanty JG, Nagababu E and Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. *Front Physiol* 2014; 5: 84. DOI 10.3389/fphys.2014.00084.
15. Gülçin İ. Antioxidant activity of food constituents: An overview. *Arch Toxicol* 2012; 86: 345–391.
16. Gülçin İ. Antioxidant properties of resveratrol: a structure activity insight. *Innov Food Sci Emerg* 2010; 11: 210–218.
17. Lannan KL, Refaai MA, Ture SK, et al. Resveratrol preserves the fonction of human platelets stored for transfusion. *Br J Haematol* 2016; 172: 794–806.
18. Bonilla E, Medina-Leendertz S, Villalobos V, et al. Paraquat-induced oxidative stress in drosophila melanogaster: Effects of melatonin, glutathione, serotonin, minocycline, lipoic acid and ascorbic acid. *Neurochem Res* 2006; 31: 1425–1432.
19. Şişecioğlu M, Çankaya M, Gülçin İ, et al. Interactions of melatonin and serotonin with lactoperoxidase enzyme. *J Enzyme Inhib Med Chem* 2010; 25: 779–783.
20. Fairbanks V and Klee G. Measurement of hemoglobin concetration in whole blood. In; text book, Clin Chem 1986; 1532–1534.
21. Stocks J, Offerman EL, Modell CB, et al. The susceptibility to autoxidation of human red cell lipids in health and disease. *Br J Haematol* 1972; 23: 713–724.
22. Khoschsorur GA, Winklhofer-Roob BM, Rabl H, et al. Evaluation of a sensitive HPLC method for the determination of malondialdehyde, and application of the method to different biological materials. *Chromatographia* 2000; 52(3–4): 181–184.
23. Paglia DE and Valentine WN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158–69.
24. Goth L. Asiple method for determination of serum catalase activation and revision of reference range. *Clin Chim Acta* 1992; 196: 143–152.

25. Fairbanks VF and Klee MD. Biochemical aspect of hematalogy. In: CA Burtis and ER Ashwood (eds) *Tietz textbook, Cilinical Chemistry*. Philadelphia: WB Saunders Company, 1994, pp.1974–2072.
26. Rickly EE, Ghazanfar SA, Gibbons BH, et al. Carbonic anhydrase from human erythrocytes. *J Biol Chem* 1964; 239: 1065–1078.
27. Wilbur KM and Anderson NG. Electrometric and colorimetric determination of carbonic anhydrase. *J Biol Chem* 1948; 176: 147–154.
28. Boztaş M, Çetinkaya Y, Topal M, et al. Synthesis and Carbonic anhydrase isoenzymes I, II, IX, and XII inhibitory effects of dimethoxybromophenol derivatives incorporating cyclopropane moieties. *J Med Chem* 2015; 58: 640–650.
29. Özgeriş B, Göksu S, Polat Köse L, et al. Acetylcholinesterase and carbonic anhydrase inhibitory properties of novel urea and sulfamide derivatives incorporating dopaminergic 2-aminotetralin scaffolds. *Bioorg Med Chem* 2016; 24: 2318–2329.
30. Nagababu E, Scott AV, Johnson DJ, et al. Oxidative stress and rheologic properties of stored red blood cells before and after transfusion to surgical patients. *Transfusion* 2016; 56: 1101–1111.
31. Nedzi M, Chabowska AM, Rogowska A, et al. Leucoreduction helps to preserve activity of antioxidant barrier enzymes in stored red blood cell concentrates. *Vox Sang* 2016; 110: 126–133.
32. Huyut Z, Şekeroğlu MR, Balahoroğlu R, et al. The Relationship of Oxidation Sensitivity of Red Blood Cells and Carbonic Anhydrase Activity in Stored Human Blood; Effect of Certain Phenolic Compounds. *BioMed Res Int* 2016; 2016: 3057384, http://dx.doi.org/10.1155/2016/3057384.
33. Ravikumar S, Hsieh C and Rajashekharaiah V. Prospects of curcumin as an additive in storage solutions: a study on erythrocytes. *Turk J Med Sci* 2016; 46: 825–833.
34. Dumaswala UJ, Zhuo L, Jacobsen DW, et al. Protein and lipid oxidation of banked human erythrocytes: role of glutathione. *Free Radic Biol Med* 1999; 27: 1041–1049.
35. Knight JA, Voorhees RP and Martin L. Lipid peroxidation in stored red cells. *Transfusion* 1992; 32: 354–357.
36. Knight JA, Voorhees RP and Martin L. The effect of metal chelators on lipid peroxidation in stored erythrocytes. *Ann Clin Lab Sci* 1992; 22: 207–213.
37. Racek J, Herynkova R, Holecek V, et al. Influence of antioxidants on the quality of stored blood. *Vox Sang* 1997; 72: 16–19.
38. Allegra M, Gentile C, Tesoriere L, et al. Protective effect of melatonin against cytotoxic actions oh malondialdehyde: an in vitro study on human erythrocytes. *J Pineal Res* 2002; 32: 187–193.
39. Gültekin F, Akdogan M, Altuntas I, et al. Changes in erythrocyte lipid peroxidation and antioxidant potential rupings storage of blood and protective effect of melatonin. *Turk J Biochem* 2000; 25: 83–91.
40. Rajashekharaiah V, Koshy AA, Koushik AK, et al. The efficacy of erythrocytes isolated from blood stored under blood bank conditions. *Transfus Apher Sci* 2012; 47: 359–364.
41. Kurata M, Suzuki M and Agar NS. Antioxidant systems and erythrocyte lifespan in mammals. *Comp Biochem Physiol B* 1993; 106: 477–487.
42. Eaton JW. Catalases and peroxidases and glutathione and hydrogen peroxide: mysteries of bestiary. *J Lab Clin Med* 1991; 118: 3–4.
43. Gülçin İ, Scozzafava A, Supuran CT, et al. Rosmarinic acid inhibits some metabolic enzymes including glutathione S-transferase, lactoperoxidase, acetylcholinesterase, butyrylcholinesterase, and carbonic anhydrase isoenzymes. *J Enzyme Inhib Med Chem* 2016; 31: 1698–1702.
44. Taslimi P, Sujayev A, Garibov E, et al. The Synthesis of new cyclic thioureas and evaluation of their metal-chelating activity, asetylcholinesterase, butyrylcholinesterase and carbonic anhydrase inhibition profiles. *J Biochem Mol Toxicol* 2017; 31. doi: 10.1002/jbt.21897.
45. Huyut Z, Beydemir Ş and Gülçin İ. Inhibition properties of some flavonoids on carbonic anhydrase I and II isoenzymes purified from human erythrocytes. *J Biochem Mol Toxicol* 2017 doi: 10.1002/jbt.21930.

46. Huyut Z, Beydemir Ş and Gülçin İ. Inhibitory effects of some phenolic compounds on the activities of carbonic anhydrase: from in vivo to ex vivo. *J Enzyme Inhib Med Chem* 2016; 31: 1234–1240.
47. Yıldırım A, Atmaca U, Keskin A, et al. N-Acylsulfonamids strongly inhibit human carbonic anhydrase isoenzymes I and II. *Bioorg Med Chem* 2015; 23: 2598–2605.
48. Genç H, Kalin R, Köksal Z, et al. Discovery of potent carbonic anhydrase and asetylcholinesterase inhibitors: 2-aminoindan β-lactam derivatives. *Int J Mol Sci* 2016; 17: pii: E1736, doi:10.3390/ijms17101736.