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Alternation in Erythrocyte Enzyme Antioxidant Activity during Blood Storage

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Abstract

Background: Blood is permanently exposed to oxidation stress and therefore has a high antioxidants capacity. Many different factors increasing the demand for the antioxidant capacity can be observed in the stored blood of donors. Consequently, damage to erythrocytes by free radicals may occur. So it is useful to control the alternation of anti-oxidant enzymes in stored blood at different days of storage. The aim of the present study was to determine the alternation of erythrocyte superoxide dismutase and glutathione peroxidase enzyme activities in stored blood.

Material and Methods: Blood samples were obtained from 67 donors with average age of 26 years. Samples were collected in CPDA-1 anticoagulation solution. Erythrocyte superoxide dismutase and glutathione peroxidase enzyme activities were determined using Kei Satoh method and kits from Randox. The measurements were performed at the days 1, 7, 14, 21, 28 and 35 of storage. The blood bags were stored after each sampling at 4□C. Data were analyzed using analytical variance statistical test and SPSS version16 software.

Results: The erythrocyte levels of erythrocyte superoxide dismutase and glutathione peroxidase enzyme activities decreased significantly at the day 14th ($P<0.001$) of storage compared to day 1.

Conclusion: Our results suggest that during blood storage, antioxidant defense in erythrocytes were depleting gradually depending on the day of storage. Based on our finding a 14 days period can be considered a safe storage limit for transfusion in relation to oxidative stress on the RBCs in storage medium.

Key words: Red blood cell, oxidative damage, antioxidant enzymes, blood storage.

Introduction

Erythrocyte is one of the important blood products that can be stored for 35-42 days in blood banks. Erythrocyte mass recipients are patients, who have a need for increasing their oxygen carrying capacity. Red blood cells transfer oxygen to all cells, but with continuous exposure to the oxidative stress. The quality of RBC products during the storage period should be maintained, but the red blood cells stored over time face changes in biophysical, biochemical and immunological specifications called RBC storage lesion [1-10]. Oxidative damage is the most important factor causing RBC storage lesion which is caused by free radicals and can affect RBC quality [11]. Free radicals can damage RBC products by lipid and protein oxidation [12-15]. The membrane of the RBC is also influenced by oxidative damage which can be an important factor for RBC hemolysis during storage.

RBCs are more exposed to oxidative stress and the risk of oxidative damage among these cells is high but their antioxidant system is also more sensitive and powerful than other cells [16]. Erythrocytes act against oxidative damage, equipped with an antioxidant system that contains enzymes: superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase. Super oxide dismutase (SOD) is the first line of defense in the cell which converts super oxide ion to hydrogen peroxide and oxygen, then catalase converts hydrogen peroxide to water and oxygen [17]. Glutathione peroxidase (GPX) belongs to the selenoproteins family and catalyses the reduction of H2O2 and hydro peroxides forms from fatty acids, there by effectively removing toxic peroxides from living cells [17]. Glutathione reductase (GR) is the enzyme which is required to maintain reduced glutathione

(GSH). The thiol groups are very reactive to a great variety of oxidant agents and are an important factor in the erythrocyte anti-oxidant system [17].

In normal physiology conditions there is a balance between RBCs' antioxidant enzymes and free radicals [18, 19], but when erythrocytes are against oxidative stress, such as being in blood storage for a long period, RBCs' antioxidant enzymes cannot protect erythrocytes against oxidative damage by free radicals and RBC storage lesion can occur due to oxidative damage [20], with a negative effect on RBC quality during storage.

In this study, we investigated antioxidant enzymes activity during RBC storage measuring GPX and SOD activity up to 35 days after the start of the blood storage in blood bank conditions.

Materials and Methods

In this study 67 whole blood bags were randomly selected from healthy donors without any infections, cardiovascular and other diseases. After collection of blood from healthy donors, the whole blood underwent screening tests such as hepatitis, HIV, and syphilis and then transferred to laboratory and stored in 2-6˚c in blood bank conditions. We measured GPX and SOD enzyme activities at the start of weeks 1, 2, 3, 4, 5 and the six (days 1, 7, 14, 21, 28 and 35) of storage in blood bank conditions (2-6˚c).

Sample preparation for measurement of SOD activity

At first, the whole blood was centrifuged at 800Xg for 10 minutes and red blood cells were separated from plasma. Then erythrocytes were washed 3 times with serum physiology (NaCl 0.9%) and additional plasma and proteins were discarded. Erythrocytes were then hemolyzed with addition of 2cc of distilled water and diluted 25 times by adding diluent solution. Auto analyzer Hitachi 902 was calibrated by SOD Randox standard solution, and samples were transferred for measurement of SOD activity in RBC using Randox kits (Cat.No. SD 125). Hemoglobin in RBCs was measured by a cell counter (Sysmex K800).

Measurement of SOD activity

The method used employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenpl)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. The super oxide dismutase activity is then measured by the degree of inhibition of this reaction (Randox Kit Cat.No.SD 125). Then the Activity of SOD is then calculated using this formula: Activity of SOD (Iu/gHb) = SOD activity (IU)/

Table 1: Super oxide dismutase activity in different weeks of blood storage.

week	SOD Average activity *u/gHb* $\bar{x} \pm SD$ n=67	P Value
First	19.18±9.5	-
second	15.34 ±7.8	P<0.68
Third	13.45±5.4	P<0.001
Forth	12.4±6.23	P<0.001
Fifth	8.8±3.8	P<0.001
Sixth	6.8±4.11	P<0.001

Hemoglobin (gr/dl) X 100

Sample preparation for measurement of GPX activity

At first 0.5cc of the whole blood was diluted with 1cc diluent solution and put in ambient temperature for 5 minutes. Then 1cc of hemoglobin solution was added and transferred to auto analyzer HITACH 902 (calibrated with GPX control blood, Randox kit RS 504).

Measurement of GPX activity

In this method, glutathione peroxidase (GPX) catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADH to NADP. The decrease in absorbance at 340 nm is measured (Randox Kit). Then the activity of GPX is calculated using this formula:

Activity of GPX (Iu/gHb) = GPX activity (IU)/ Hemoglobin (g/dl)

Statistical analysis

In this study, data analysis was performed using SPSS (version 16) program. P values $P<0.05$ were considered significant.

Results

A significant decrease in SOD enzyme activity was seen after 2 weeks of blood storage (Table 1). According to our results, GPX enzyme activity significantly decreased after just one week of blood storage (Table 2).

In the present study, we measured the percentage of decreasing antioxidant enzyme activity during blood storage for 35 days. According to our results, after 2 weeks of blood storage, SOD enzyme activity decreased 20% compared with the first day (Figure 1); also GPX enzyme activity decreased 33% after 2 weeks of storage compared with the first day (Figure 2). At the end of the study (35 days of blood storage), SOD enzyme activity in erythrocytes decreased as much as 65% and GPX enzyme activity decreased as much as 68% compared with SOD and GPX enzyme activity in the first day.

Discussion

Human RBCs stored in standard blood banking conditions undergo time dependent changes, including loss of ATP, GSH, lipids and structural and membrane proteins, possibly leading to decreased

Table 2: Glutathione peroxidase activity in different weeks of blood storage.

week	GPX average activity u/gHb $\bar{x}$ ±SD n=67	P Value
First	40.6±14.7	-
second	27.25±12.9	P<0.001
Third	16.7±7.8	P<0.001
forth	15.4±11.5	P<0.001
Fifth	14.6±10.3	P<0.001
Sixth	12.9±15.5	P<0.001

Figure 1: Super oxide dismutase activity in different weeks of blood storage.

deformability and unsatisfactory post transfusion in vivo survival. One of important changes that occur during storage is oxidative damage from free radicals that is due to imbalance between the production of reactive oxygen species and the efficiency of the antioxidant defense [21]. This is an important factor for RBC lesion that can affect an RBC quality during storage. Anti-oxidant system keeps free radicals at physiologically optimal levels and acts as an inhibitor of oxidation and deactivates free radicals [22, 23]. SOD, CAT and GPX are three main enzymatic systems of the organism against free radicals and peroxides [12, 24].

SOD plays an important role in the protection of cells against the deleterious effect of free radicals by converting super oxide anion to hydrogen peroxide, which is then transformed to water by GPX or by catalase. In erythrocytes, catalase and GPX jointly protect hemoglobin from oxidative damage. GPX catalyses the reduction of hydrogen peroxide by reduced glutathione (GSH) and protects hemoglobin from oxidative breakdown [22].

According to our results, GPX activity in RBCs significantly decreased after one week of storage, also SOD enzyme activity in RBC significantly decreased after two week of RBC storage. Our results showed that GPX appears to provide the primary anti-oxidant defense in storaged RBCs. Our results are similar to those of Marjani et al. [25], who reported decreased SOD and GPX activity at days 7-11 of storage. Decrease in GPX activity, concurrent with an increase in oxidative modification of membrane lipids and proteins, may destabilize the membrane skeleton, thereby compromising RBCs' survival.

The erythrocyte anti-oxidant defense potential decreases in correlation with the increase of oxidative stress. Our results are similar to those of Aslan et al.[23] who reported that enzyme activity of GPX and SOD, significantly decreases after 9 days and 13 days of erythrocytes storage respectively. Reduction of enzyme activity continued until the 6th week of storage. Also Jozwik et al.[7] reported that a 12 day period can be considered a safe storage limit for RBC storage which is in line with our findings. Ogunro et al. [8] reported decreased GPX and SOD activity by 17.1 % on day 15 and they concluded that a 15 days period can be considered a safe RBC storage period. Gultekin et al. [24] observed that the enzyme activity of SOD, GPX and catalase decreased after 7-14 days of storage. According to our results, SOD enzyme activity decreased 20% and GPX activity decreased 33% in the second week of RBC storage. These results are in accordance with the results of other researchers. Thus, we could estimate that the equilibrium between anti-

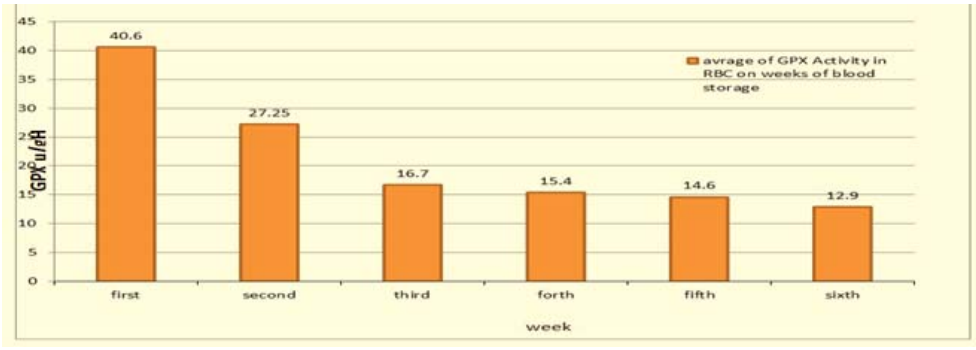

Figure 2: Glutathione peroxidase enzyme activity in different weeks of blood storage.

oxidant defense and alternation agents could break after 14 days of storage, irretrievably with great negative consequences on cellular functions and morphology which could result in aging and even lysis of RBCs.

Based on our results the best duration for blood storage seems to be is up to two week after donation. In this period, changes of SOD were not significant and it seems, there is a balance between oxidant and anti-oxidant mechanisms. Based on our results we suggest that the storage of erythrocytes, for more than 14 days causes a significant decrease of blood quality.

Conclusion

Our results suggest that during blood storage, antioxidant defense in erythrocytes were depleting gradually depending on the day of storage. Based on our finding a 14 days period can be considered a safe storage limit for transfusion in relation to oxidative stress on the RBCs in storage medium.

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