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Alterations in Plasma Lipid Peroxidation and Erythrocyte Superoxide Dismutase and Glutathione Peroxidase Enzyme Activities During Storage of Blood

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Abstract: The present study was designed to determine the alteration of plasma lipid peroxidation and erythrocyte Superoxide Dismutase and Glutathione Peroxidase enzyme activities in stored blood. This study is carried out to find out the quantitative alterations and the useful length of stored blood. The whole blood were taken from 10 donors. Red Blood Cells (RBC) were counted in whole blood. The levels of Potassium (P) and lactate dehydrogenate activity (LDH) were measured in plasma for determination of hemolysis. The plasma levels of malondialdehyde (MDA) and erythrocyte Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) were studied for determination of lipid peroxidation and antioxidant enzyme activities, respectively. The measurement were performed at the day 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35 of the storage. The plasma levels of malondialdehyde (MDA) and Potassium and lactate dehydrogenate activity increased ($p<0.05$) depending on storage time whereas erythrocyte Superoxide Dismutase and Glutathione Peroxidase enzyme activities and Red Blood Cells decreased ($p<0.05$). The alterations of MDA, SOD, GPx, P, LDH and RBC in the measurement days were as follows: MDA, P and LDH significantly increased at the day 9, 5 and 5 whereas SOD, GPx and RBC decreased at the day 11, 7 and 29, respectively. These results suggest that increased level of MDA and decreased level of SOD and GPx in the stored blood can not improve the vialibilty and longevity of RBC by increasing cell damage caused by free radicals at the days 7-11. To improve the quality of stored blood is to supplement blood donors with antioxidants and vitamins at least one week before blood collection. We conclude that increased level of MDA and decreased SOD and GPx in stored blood which can cause the beginning of hemolysis. It is therefore necessary to control these factors before blood transfusion.

Key words: Lipid peroxidation, superoxide dismutase, glutathione peroxidase

Introduction

It is generally believed that membrane lipids are major targets for cellular damage induced by free radicals. Red blood cells offer a number of advantages studies of effects of oxidants on protein and lipid breakdown and oxidation respectively. Oxygen radicals are produced continually in red cells by hemoglobin auto-oxidation (Misra and Pridovich, 1972; Hebbel *et al*., 1982). Free radicals may cause lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde) and damage

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macromolcules and cellular structure of the organism, endothelium and erythrocytes. Plasma malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to definite oxidation of polyunsaturated fatty acids such as linoleic and linolenic acid and thus serves as a reliable marker of lipid peroxidation (Boaz *et al*., 1999; Fiorillo *et al*., 1998). Free radicals are eliminated from the body by their interaction with non-enzymic and enzymic antioxidants such as uric acid, albumin, bilirubin, vitamins E, C, A, glutathione, glutathione peroxidase, superoxide dismutase and catalase (Kohen *et al*., 1996).

There are a few reports describing the use of stored blood without any alteration in biochemical factors. In a study Acid-Citrate-Dextrose was used as preservative factor in stored blood (Gibson *et al*., 1947; Ross *et al*., 1947). In other study, Citrate-phosphate-Dextrose and Citrate-phosphate-Dextrose-Adenin were used as blood preservative factor in 1957 and 1960, respectively. In further study (1978) glucose was added to the final constituent and the CPDA-1 was formed and the useful blood storing time increased to 35 days which with adding of Mannitol to CPDA-1, this time increased to 42 days (Orlina and Josephson, 1969). On the other hand there are some reports about reducing of Red Blood Cell life span and antioxidant status in the stored bloods which shows there are some changes on plasma level free radicals and antioxidants. Blood is permanently exposed to oxidation stress and therefore it has a high antioxidant capacity (Lewin and Popov, 1994). In the stored blood of donors many factors increasing the demands on the antioxidant capacity can be observed. Consequently, damage to erythrocytes by free radicals may occur. This can be manifested in different ways. Potassium and Lactate dehydrogenase release from erythrocytes into plasma can be considered a very good indicator of the oxidative damage of erythrocyte membranes. Malondialdehyde (MDA) may serve as a marker of lipoperoxidation (Niki *et al*., 1988) while sufficient activity of enzyme Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) protects the blood against oxidative damage (Yamagchi *et al*., 1992). It is useful to control the alteration of antioxidant enzymes and lipid peroxidation in stored blood at the different days. For this reason the aim of the present study was designed to determine the alteration of plasma lipid peroxidation (by measuring the level of MDA) and erythrocyte Superoxide Dismutase and Glutathione Peroxidase enzyme activities in stored blood. This study is carried out to find out the quantitative alterations and the useful length of stored blood.

Materials and Methods

Blood samples were obtained from 10 donors who had not received drugs and vitamins. Bloods were collected in CPDA-1 anticoagulation solution. People were chosen from the people refereed to the Blood Transfusion Center in Gorgan City. This study was carried out during 2006. Health of blood donor people were defined as not having a major medical illness, no hospital admissions, no current medication, no physical activity, no smoking and a subjective perception of good health as determined by health questionnaire. Red Blood Cell were counted in whole blood. After Red Blood Cell counting, whole blood of each donors were centrifuged at 4000× g for 10 min and the plasma was obtained. Plasma potassium level and lactate dehydrogenase activity were measured for determination of hemolysis. The plasma malondialdehyde (the level of lipid peroxidation expressed as malondialdehyde [MDA]) and erythrocyte Superoxide Dismutase and Glutathione Peroxidase enzyme activities were determined with Kei Satoh method and kits from Randox, respectively (Satoh, 1978; Woolliams *et al*., 1983; Paglia and Valentine, 1967) and spectrophotometry techniques (model JENWAY 6105 UV / VIS) in the Laboratory of Biochemistry (Faculty of Medicine). The level of potassium and lactate dehydrogenase activity were determined with a FLM3 flame photometer (Radiometer Copenhagen) and commercial kit, respectively. The measurement were performed at the day 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35 of the storage. The blood bags were stored after each sampling at 4°C. Data was analyzed by analytical variance statistical test, using SPSS-10 software. $p<0.05$ was considered significant.

Malondialdehyde Measurement

To 0.5 mL plasma, 2.5 mL of trichloroacetic acid is added and the tube is left to stand for 10 min at room temperature. After centrifugation at 3500 rev. min^{-1} for 10 min, the supernatant is decanted and the precipitate is washed once with sulfuric acid. Then 2.5 mL sulfuric acid and 3 mL thiobarbituric acid (TBA) in sodium sulfate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling water bath for 30 min. After cooling in a cold water, the resulting chromogen is extracted with 4 mL of n-butyl alcohol by vigorous shaking. Separation of the organic phase is facilitated by centrifugation at 3000 rev. min^{-1} for 10 min and its absorbance is determined at the wavelength of 530 nm.

Results

The plasma levels of malondialdehyde (MDA) and Potassium and lactate dehydrogenate activity increased ($p<0.05$) depending on storage time whereas erythrocyte Superoxide Dismutase and Glutathione Peroxidase enzyme activities and Red Blood Cells decreased ($p<0.05$). The alterations of MDA, SOD, GPx, P, LDH and RBC in the measurement days were as follows: MDA, P and LDH significantly increased at the day 9, 5 and 5 whereas SOD, GPx and RBC decreased at the day 11, 7 and 29, respectively (Table 1).

Discussion

Exposure of red blood cells to oxygen radicals can induce hemoglobin damage and stimulate lipid peroxidation and hemolysis. Metabolism of erythrocyte antioxidants are very important for red blood cells. Lipid peroxidation can cause cellular damage, death and diseases. Some researchers showed that lipid peroxidation in creased in some diseases such as Thalassemia major, Glucose-6-phosphate dehydrogenase deficiency, auto immune hemolytic anemia and during the ageing of erythrocyte (Hochstein and Jain, 1981). Formation of free radicals in stored blood is of a complex origin. The high concentration of polyunsaturated fatty acids, hemoglobin mediated formation of the hydroxyl radical from hydrogen peroxide and other changes should be mentioned

Table 1: Plasma Malondialdehyde (MDA), Lactate dehydrogenase (LDH), erythrocyte Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and red blood cells (RBC)

Time (Day)	MDA (n mol mL^{-1})	SOD (U g^{-1}Hb)	GPx (U g^{-1} Hb)	LDH (U L^{-1})	K (m mol L^{-1})	RBC (million μL^{-1})
0	3.69±0.014	1236.40±5.31	34.15±1.10	381.70±0.82	4.20±0.008	4.99±0.140
1	3.69±0.011	1234.0±5.730	33.85±1.02	382.0±0.810	4.21±0.014	4.98±0.100
3	3.71±0.019	1233.80±5.47	32.93±1.24	382.70±0.82	4.22±0.015	4.91±0.060
5	3.72±0.011	1231.80±7.96	32.59±1.42	516.10±2.51*	5.21±0.014*	4.90±0.040
7	3.72±0.012	1231.50±6.29	27.25±0.83*	564.20±5.92	6.93±0.019	4.87±0.040
9	5.46±0.141*	1231.0±3.160	26.84±0.64	597.0±2.010	7.19±0.10	4.87±0.040
11	5.59±0.178	856.0±36.20*	26.56±0.78	608.50±4.62	7.94±0.01	4.88±0.030
13	5.67±0.135	851.30±32.60	26.08±0.94	621.10±7.35	8.27±0.02	4.83±0.040
15	5.70±0.123	846.50±32.33	25.84±0.69	629.10±8.08	9.18±0.03	4.81±0.040
17	5.92±0.093	846.50±32.33	25.88±0.57	635.0±7.300	9.55±0.07	4.76±0.030
19	6.03±0.103	833.70±18.89	25.71±0.53	648.80±2.74	10.13±0.04	4.74±0.010
21	6.74±0.010	829.90±29.74	25.48±0.51	659.80±3.82	11.97±0.06	4.73±0.030
23	6.80±0.040	829.20±21.38	25.35±0.48	668.10±6.36	12.17±0.13	4.70±0.040
25	6.84±0.051	828.30±29.03	25.13±0.42	677.20±6.71	14.14±0.10	4.67±0.010
27	6.90±0.047	825.40±29.20	24.80±0.42	695.92±4.64	15.09±0.08	4.12±0.010
29	6.93±0.043	822.90±11.14	24.65±0.38	699.93±1.38	17.33±0.15	3.80±0.040*
31	6.96±0.065	817.30±9.950	24.47±0.37	730.83±4.10	18.37±0.27	3.76±0.030
33	7.0±0.1050	815.50±13.79	24.31±0.40	748.20±7.74	19.79±0.31	3.68±0.030
35	7.10±0.134	811.90±12.37	24.19±0.31	774.40±10.18	20.78±0.50	3.60±0.030

*$p<0.05$ at the days mentioned above

(Knight *et al*., 1991; Knight *et al*., 1992; Chiu *et al*., 1989; Jain, 1988). Blood also rich in esterified cholesterol. polyunsaturated fatty acids and esterified cholesterol can easily covered into lipid hydro peroxides which are unstable. Lipoperoxidation continues up to the formation of MDA (Knight *et al*., 1992). Malondialdehyde is an indirect marker of lipid peroxidation. MDA can modify proteins and, together with the changes of membrane lipids during lipid peroxidation, may be the main cause of damage to erythrocyte membranes and subsequent hemolysis (Knight *et al*., 1992; Chiu *et al*., 1989; Jain, 1988). An important advantage of this study when compared with those already published was the number of different storage days of blood.

The results of this study show that the plasma levels of malondialdehyde (MDA) and Potassium (P) and lactate dehydrogenate (LDH) activity increased ($p<0.05$) depending on storage time whereas erythrocyte Superoxide Dismutase and Glutathione Peroxidase enzyme activities and Red Blood Cells decreased ($p<0.05$). The alterations of MDA, SOD, GPx, P, LDH and RBC in the measurement days were as follows: MDA, P and LDH significantly increased at the day 9, 5 and 5 whereas SOD, GPx and RBC significantly decreased at the day 11, 7 and 29. Some studies showed that there are an increase of lipid peroxidation in stored blood (Knight *et al*., 1992; Aslan *et al*., 1997; Racek *et al*., 1997). Some investigators have studied stored blood related changes in lipid peroxidation and antioxidant system during storage of blood, but the results are controversial (Aslan *et al*., 1997; Racek *et al*., 1997; Korgun *et al*., 2001; Lippa *et al*., 1990; Jozwik *et al*., 1997).

Aslan *et al*. (1997) showed that MDA levels increased significantly during the storage period from day 3 to day 19 and after that, stayed unchanged. The glutathione peroxidase activity significantly decreased after day 9 and superoxide dismutase decreased after day 13.

Korgun *et al*. (2001) showed the increasing of lipid peroxidation and decreasing of antioxidant enzymes during the storage period. Theyconsidered that antioxidant system in erythrocytes might be depleted during long storage in blood bags.

Lippa *et al*. (1990) considered that MDA shows a decreasing level until the third week and the risen rapidly to very high values. They showed that a storage time not exceeding 21 days gives the best transfusion results. Jozwik *et al*. (1997) were determined antioxidant enzymes activities on days 1, 3, 7, 12, 16, 20 and 25 days of storage. They showed that the antioxidant enzymes, glutathione peroxidase (30%) and superoxide dismutase (over 10%) decreased during storage time. Their results showed that a 12 day period can be considered a safe storage limit. Gultekin *et al*. (2000) were determined the level of thiobarbituric acid reactive substance (another marker for measuring of lipid peroxidation) and antioxidant potential on days 0, 1, 3, 5, 7, 10, 14, 21, 29 and 35 of the storage. They showed that the level of thiobarbituric acid reactive substance, LDH and P increased significantly during the storage period from day 7, 3 and 5, respectively. Antioxidant potential and RBC decreased significantly during the storage period from day 14 and 28, respectively. The results of this study are in agreement with the results of studies showing that plasma levels MDA and antioxidant system is significantly increased and decreased, respectively (Korgun *et al*., 2001; Aslan *et al*., 1997; Gultekin *et al*., 2000). But present results are not in agreement with the other studies (Aslan *et al*., 1997; Lippa *et al*., 1990). Present results showed that a 7-11 day period can be considered a safe storage limit. A possible explanation for this is that the free radicals are continuously produced by haemoglobin, on the result of auto-oxidation, therefore the red blood cells are constantly exposed to the oxidative stress, but the antioxidant system can eliminate such oxidant in red blood cells. Because cellular membranes house the production apparatus of these radicals and because membranes (rich in polyunsaturated fatty acids) suffer great damage from these radical. Modification of membrane lipids has been proposed to play a major role in the process of membrane damage during blood storage (Chiu *et al*., 1982; Chiu and Claster, 1988).

Possible sources of elevated free radicals in stored blood include increased production of radical oxygen species, especially from lipid peroxidation processes and decreased antioxidant defense systems. We conclude that increased level of MDA and decreased SOD and GPx in stored blood which

can cause the beginning of hemolysis. It is therefore necessary to control these factors before blood transfusion. It appears the administration of antioxidants and vitamins to blood donors before blood sampling improves the blood quality from the point of view of deleterious actions of free radicals (Maeda *et al*., 1992). Blood of donors given antioxidants and vitamins before blood donation would be useful for total blood exchange in newborns (Lindeman *et al*., 1992) and for some patients receiving intensive care treatment.

To improve the quality of stored blood is to supplement blood donors with antioxidants and vitamins at least one week before blood collection to keep and store the stored blood for longer time until using for transfusion.

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