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Effects of Different Doses of Gamma Rays and Ascorbic Acid Concentration on Human RBCs for Conservation Purpose

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Abstract

Background Blood preservation and the development of sterile collection sets made possible the developments in blood components preparation, storage and transfusion that we have in today's blood banks and transfusion services.

Objective To investigate the effects of gamma irradiation, ascorbic acid and the combined effect of both on the lifespan of erythrocytes through determining red blood cells hemolysis for conserving it as long as possible without any change in erythrocytes biophysical properties.

Methods The blood was drawn from 10 healthy (5 males and 5 females) volunteers. Sample has been irradiated using 137Cs source. Different concentrations of ascorbic acid were used as an anti-oxidative agent for erythrocytes in blood suspension samples. A spectrometer was used for recording the data.

Results The results showed that 25% of RBCs hemolysis occurred after irradiation with 5Gy of gamma ray during 5th week of storage time while in un-irradiated sample 33.8% of RBCs hemolysis occurred during the 5th week. 25% of RBCs hemolysis for 10 µM of ascorbic acid concentration started after 7th week while for control started after 4th week. The minimum rates of RBCs hemolysis observed in the samples which pre-treated with (7 and 10) µM concentrations of ascorbic acid then irradiated with 1 Gy.

Conclusion The results indicated that irradiation of human blood with a certain doses of gamma ray, treated with small concentration of ascorbic acid or both, the two factors together can protect the blood from hemolysis for a longer time and the minimum rate of red blood cells hemolysis was observed for 10 µM ascorbic acid concentration then irradiation to 1 Gy of gamma ray.

Keywords Gamma ray, ascorbic acid, blood storage, red blood cells, oxidative damage

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List of abbreviations: ATP = Adenosine triphosphate, DPG= Biophosphoglyceric, GVHD = Graft-versus-host disease, Hb = Hemoglobin, K = Potassium, Na = Sodium, OH = Hydroxyl, RBCs = Red blood cells, SOD= Superoxide dismutase

Introduction

There is a growing concern about the possible health effects associated with exposure to electromagnetic fields.

Due to the insufficient number of blood donors in the suitable time, the process of blood conservation has become a necessary and an inevitable process. The preserved red blood cells (RBCs) for clinical transfusions must meet minimum requirements to the stored blood to continue its metabolic functions and deplete the metabolites necessary to maintain RBCs viability and function.

It is so significant to recognize that RBCs undergo structural and morphological changes

associated with Adenosine Triphosphate (ATP) depletion and oxidative damage of membrane lipids upon storage; such lesions are called storage lesions that depend on storage conditions, storage length and additives used in blood productions [1]. For this, the whole blood used for transfusion can be stored in blood bags at 4 °C, and used within approximately one month to achieve its vital goal.

Mammalian RBCs do not have nucleus like other cells in the body, therefore during their circulation through capillaries they get deformed [2]. This deformation of RBCs is one of properties to maintain viability. If the deformation of cells is too large then the cells will get hemolysis easily. The cytoskeleton structures of the RBCs membranes responsible to restore its shape after deformation by the capillaries [2]. Exposure to ionizing radiation can kill some types of cells while modify others [3].

For checking the validity of stored blood has been investigated by (Marjani et al. 2007) and (Huyut et al. 2016). These authors investigated the malondialdhyde formation and antioxidant enzyme activity in stored blood [4,5]. Reactive oxygen metabolites and free radicals are generated normally in aerobic organisms. Membrane lipids are major targets for cellular damage of radical mediated lipid peroxidation. It is recognized that aerobic cells are primary protected from the free radical damage by endogenous antioxidants [6]. The effect of gamma radiation on the stored blood was investigated in several studies using wide range of doses. Moor and Ledoford (1985) who studied the effect of 40 Gy irradiation on the in-vitro storage properties of packed red cells [7], Anand et al. (1997) from zero to 50 Gy [8], and Brugnara and Churchill (1992) 2000 cGy [9]. The prevention of graft - versus – host disease (GVHD) is probably one of the major reasons for blood irradiation, and its use in susceptible patients has increased [10,11].

Many studies; Britten (1999), Davey et al. (1992), Pribush et al. (1994) and Katz et al. (1996) indicated that, as the radiation sensitivity of T lymphocyte is different widely from that of red blood cells a high enough dose should be applied to destroy almost all the T lymphocytes while causing as little as possible damage to the red cells [12-15]. Recent studies indicate that doses higher than 30 Gy, are required [16]. Since it causes only minor changes in the concentration of the essential constituents of the red cells, e.g. ATP, biophosphoglyceric (DPG) catalase, glutathione peroxides and superoxidedismutase (SOD). However, these higher doses cause potassium leakage from the red blood cells which is attributed to the damage occurred in the cell membrane. In the range of doses used (10-30) Gy, the effect of irradiation when RBCs are stored at 4 °C is to promote a balanced passive exchange of intracellular Potassium (K) for extra cellular Sodium (Na); this exchange does not affect the volume of the RBCs [9]. Radiation damage to RBCs occurs as the hydroxyl (OH) radicals can react with other cellular components in addition to the target molecule.

The aim of this study was to investigate the effects of gamma radiation, ascorbic acid (vitamin c) added in small concentrations, and the combined effect of both on the lifespan of erythrocytes through determining RBCs hemolysis for conserving it as long as possible without any change in erythrocytes biophysical properties.

Methods

A volume of 2.9 ml of blood was drawn from 10 healthy volunteers (5 males and 5 females) with ages between 30-40 years and collected in EDTA tubes.

Gamma irradiation

Blood samples were exposed to different doses of gamma radiation (1, 5, 10, 20 and 30 Gy) and stored at 4 °C. Gamma source was standard from 137Cs source (0.66) MeV (model GB-150 type B). The exposure rate of 0.34 Gy/min. has been calibrated by standard ionization chamber type (NE-2571) of volume 0.06 cc with air kerma, Calibration factor NK=41.0+0.25 mGy/min. measured by electrometer type NE2571/1 manufactured by nuclear enter press

Ltd in UK. The value of absorbance (RBCs hemolysis) was recorded for each dose and repeated weekly up to 10 weeks.

Adding ascorbic acid

0.1 ml of ascorbic acid with different concentrations (1, 3, 5, 7, and 10) µM of ascorbic acid was added to 2.9 ml of blood and stored at 4 °C. Each week 0.05 ml of blood was added to 9 ml of NaCl saline in a tube then 3 mL from the suspension was taken in a standard curette of the spectrophotometer, after shacking gently and carefully the value of absorbance at 577 nm was recorded. This process was repeated each week up to ten weeks.

Ascorbic acid with gamma irradiation

Different concentrations of ascorbic acid (1, 3, and 5 µM) were added into different blood samples and exposed to 20 Gy of gamma radiation. Also, the two (7 and 10 µM) concentrations of ascorbic acid added in to another blood samples then exposed to 1 Gy of gamma radiation, the samples stored at 4 °C and examined for 10 weeks.

The data were recorded using spectrometer model JASCO (V-530), UV/VIS in Japan. The intensity of the peak at 577nm of absorbance spectrum represented the degree of hemoglobin breakdown (degree of hemolysis) [17].

Statistical analysis

The results were presented as mean ± Standard deviation (SD) of percentage and statistical analysis were performed using students t-test (paired and unpaired two tailed) taking ($p < 0.01$) as the significance.

Results

Table (1) shows the percentage of hemolysis of RBCs in the case of control (without irradiation or adding the ascorbic acid) through 10 weeks; in this case we observed that the hemolysis increased as the storage time increased.

Table 1. The relation between absorbance values (RBC hemolysis) for control samples and storage times through 10 weeks at 4 °C

Storage time in weeks	Percentages of RBC hemolysis ±SD
1	7.0±1.49
2	13.0±1.34
3	18.5±1.49
4	25.0±1.49
5	33.8±1.55
6	39.0±1.49
7	44.9±1.17
8	50.2±1.49
9	56.3±1.20
10	63.7±1.63

The effect of different doses of gamma radiation (1, 5, 10, 20, and 30) Gy on the RBCs hemolysis of the blood samples are shown in table (2). It was found that irradiation of the blood samples increased RBCs hemolysis, which was undesirable, but with increasing the storage time the rate of the hemolysis decreased in all samples exposed to gamma radiation compared with the controls. It was observed that 25% of RBCs hemolysis occurred after irradiation with 5 Gy during the fifth week, while in un-irradiated sample 33.8% of RBCs hemolysis occurred during the fifth week, the difference was significant ($p<0.01$) between them.

Table 2. The relation between RBC hemolysis for blood samples exposed to different doses of gamma radiation and stored at 4 °C through 10 weeks

Gamma radiation dose Gy	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
1	4.0± 1.22	8.0±1 .26	13.0± 1.56	16.0± 1.67	20.0± 1.58	23.9± 1.25	27.6± 1.42	30.0± 1.43	32.4± 1.67	34.7± 1.37
5	6.4± 1.19	9.2± 1.11	15.0± 1.21	21.2± 1.56	25.0± 1.87	29.5± 1.73	33.0± 1.63	36.3± 1.69	38.2± 1.72	41.1± 1.24
10	6.8± 1.33	10.0± 1.55	15.9± 1.48	22.5± 1.59	26.0± 1.23	31.0± 1.20	34.7± 1.31	38.0± 1.40	41.2± 1.73	43.4± 1.82
20	7.2± 1.87	12.0± 1.17	17.3± 1.28	24.0± 1.51	27.5± 1.29	32.0± 1.62	35.4± 1.77	39.0± 1.30	42.2± 1.14	45.0± 1.32
30	7.5± 1.15	13.5± 1.43	19.0± 1.23	25.0± 1.49	28.9± 1.54	34.0± 1.17	37.2± 1.40	41.8± 1.53	44.9± 1.61	47.8± 1.31

The results of the blood samples treated with different ascorbic acid concentrations (1, 3, 5, 7 and 10) µM are shown in table (3). The results showed that 25% of RBCs hemolysis started after 7th week while in untreated one RBCs hemolysis reaches 25% after 4th week only. 25% of RBCs hemolysis for 10 µM of ascorbic acid concentration started after 7th week while for control started after 4th week, this difference was significant ($p<0.01$).

Table 3. The relation between RBC hemolysis for blood samples treated with different concentrations of ascorbic acid and storage times through 10 weeks at 4 °C

concentrations of ascorbic acid µM	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
1	1.0± 1.23	2.0± 1.34	3.0± 1.40	4.3± 1.28	5.7± 1.32	8.0± 1.65	10.0± 1.54	12.0± 1.48	14.2± 1.39	16.7± 1.46
3	1.3± 1.26	2.6± 1.87	4.2± 1.94	6.3± 1.82	8.4± 1.25	10.0± 1.67	13.0± 1.97	15.3± 1.60	18.0± 1.59	21.3± 1.33
5	1.9± 1.11	3.0± 1.19	7.0±1 .61	11.6± 1.75	14.0± 1.83	17.0± 1.54	19.4± 1.20	21.9± 1.16	23.0± 1.22	25.0± 1.43
7	4.0± 1.19	9.8± 1.41	12.0± 1.49	15.2± 1.62	17.7± 1.58	20.1± 1.67	22.5± 1.92	25.0± 1.44	27.2± 1.88	29.5± 1.73
10	5.2± 1.13	10.4± 1.56	13.7± 1.37	17.2± 1.47	20.4± 1.73	22.9± 1.94	25.0± 1.76	27.0± 1.57	29.3± 1.53	33.0± 1.82

The changes in percentages of RBCs hemolysis with respect to the storage time (through 10 weeks) were studied for blood samples pre-treated with (1,3 and 5) µM and (7 and 10) µM then irradiated with 20 Gy and 1 Gy respectively, the results are shown in tables (4 and5). In pre-treated with (1, 3 and 5) µM concentrations of ascorbic acid then irradiated to 20 Gy, we observed that 25% of RBCs hemolysis for 3µM concentration of ascorbic acid started at 5th week while for 1µM this ratio of hemolysis started between 6th and 7th weeks as shown in table (4).

Table 4. The relation between percentages of RBC hemolysis for blood samples treated with low ascorbic acid concentrations then exposed to 20 Gy dose of gamma radiation and storage times through 10 weeks at 4 °C

Blood samples treated with ascorbic acid and exposed to 20 G	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
1	3.0± 1.23	7.9± 1.1	14.0± 1.24	17.5± 1.26	20.4± 1.38	23.8± 1.57	27.6± 1.68	30.0± 1.75	32.2± 1.89	33.2± 1.66
3	4.8± 1.23	9.6±1 .45	14.4± 1.34	20.2± 1.33	25.0± 1.67	27.5± 1.42	30.0± 1.56	33.3± 1.38	35.2± 1.74	36.8± 1.56
5	6.7± 1.21	10.0± 1.35	15.2± 1.60	21.5± 1.43	26.0± 1.49	28.9± 1.65	31.7± 1.23	34.5± 1.72	36.2± 1.56	38.4± 1.29

The minimum rates of RBCs hemolysis observed in samples which pre-treated with (7 and 10) µM concentrations of ascorbic acid then irradiated with 1 Gy as shown in table (5), in this case 25% of RBCs hemolysis started at 9th week.

Table 5. The relation between percentages of RBC hemolysis for blood samples treated with different high ascorbic acid concentrations then exposed to 1 Gy dose of gamma radiation and storage times through weeks at 4 °C

Blood samples treated with a ascorbic acid and exposed to 1 Gy	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
7	2.3± 1.31	5.5± 1.82	7.8± 1.32	12.0± 1.59	15.5± 1.42	18.4± 1.63	20.0± 1.49	22.0± 1.24	24.0± 1.28	26.5± 1.26
10	1.8± 1.16	4.0± 1.22	6.2± 1.43	10.0± 1.38	13.0± 1.12	15.5± 1.29	17.6± 1.61	20.0± 1.52	22.0± 1.18	24.0± 1.38

Discussion

The validity of stored blood has been investigated by different studies and the current study was done to show the effects of gamma radiation, ascorbic acid added in small concentrations, and the combined effect of both on the lifespan of erythrocytes through determining RBCs hemolysis for conserving it as long as possible without any change in erythrocytes biophysical properties. As shown in table (1), the percentage of hemolysis of RBCs in the case of control increased as the storage time increased through 10 weeks due to the elevation of lipid peroxidation and hemoglobin oxidation [8] and extracellular potassium (K) concentration in red blood cells storage [18].

In irradiation of blood samples to different doses of gamma radiation (1, 5, 10, 20, and 30) Gy, it was found that RBCs hemolysis increased but with increasing the storage time the rate of the hemolysis decreased in all samples compared with the controls. During the fifth week, it was observed that 25% and 33.8% of RBCs hemolysis occurred after irradiation with 5Gy and in un-irradiated sample respectively, and the difference was significant ($p<0.01$) between them as shown in table (2). Mintz and Anderson (1993) found that the mean Potassium (k) and hemoglobin (Hb) concentration at the end of 35 days of storage for both the irradiated group (30 Gy) and the un-irradiated one were not significantly different [19].

Treated the blood samples with different ascorbic acid concentrations (1, 3, 5, 7 and 10) µM showed the role of antioxidant effect of ascorbic acid which increased with increasing the concentrations up to 10µM. Results of this study match the results that by Lenton et al (2003), who affirmed that ascorbic acid plays a vital and central role in the defense against free radicals and oxidants that are implicated in chronic diseases [20].

Different effects were obtained in pre-treated with of ascorbic acid then irradiated to gamma ray. The minimum rates of RBCs hemolysis observed in samples which pre-treat high concentrations of ascorbic acid then irradiated with 1 Gy. The results indicated that with increasing ascorbic acid concentration low dose of gamma radiation required to obtain minimum RBCs hemolysis.

The obtained results indicated that irradiation of human blood with a certain doses of gamma ray, treated with small concentration of ascorbic acid or both, the two factors together can protect RBCs of blood from hemolysis for a longer time. The minimum rate of RBCs hemolysis was observed for 10 µM ascorbic acid concentration with irradiation to 1 Gy of gamma ray. We observed when the concentration of the ascorbic acid in blood samples increased; low levels of gamma irradiation were added to obtain the minimum RBCs hemolysis.

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Author contribution

Dr. Faraj: writing the article with resolving the data. Abdullah and Dr. Muhammad performed the practical part of the work.

Conflict of interest

None.

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