EFFECT OF GAMMA-RAY ON THE STABILITY OF STORED RBCs MEMBRANE

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Abstract

Blood bank stored RBCs were reported to undergo several changes including RBCs membrane protein changes. The present study was designed to investigate the responses of stored RBCs in term of membrane stability of cells to γ-ray. Stored RBCs for 15 days and 30 days was associated with a significant decrease in the stability of RBCs relative to (fresh RBCs). In addition, 1Gy gamma radiation did not change the stability of fresh or stored RBC. In contrast, 10Gy gamma radiation was associated with a further significant decrease in the stability of 15 days stored RBCs relative to (fresh RBCs). However, an apparent significant increase in the stability was observed for the RBCs stored for 30 days and irradiated by 10Gy gamma radiation.

Introduction

Normally, human red blood cells (RBCs) circulate in the blood stream for 110-120 days [1], and the senescent RBC are eliminated by phagocytic cells of the reticuloendothelial system (RES), primarily in the spleen but also in the liver and bone marrow [2].

The erythrocyte or red blood cell (RBC) membrane consists of two domains, a lipid bilayer and the cytoskeleton. The lipid domain is composed nearly equal parts of lipids and proteins, the main lipids are cholesterol and phospholipids. The proteins in the lipid domain usually extend from inside of the erythrocyte to the outside[3]. The normal membrane skeleton appears to play key role in determining most of the material properties and that it confers the extreme elasticity and resistance to yield that enable the red cell to undergo marked deformation without membrane fragmentation (loss of its stability)[4]. The erythrocyte cytoskeleton consists of several proteins that form a filamentous network under the lipid bilayer. The network is composed of spectrin, ankyrin, actin, and protein 4.1 [5].

The red cell membrane behavior under stress is a complex one, and its response depends upon the duration and magnitude of applied shear stress. It has been estimated that the isolated lipid bilayer of the RBC exhibits almost no elastic behavior[6]. The elastic shear modulus (the magnitude of force needed to induce uniaxial deformation at constant area) for the RBC membrane is not due to lipid portion of the membrane but it depends on protein components of the membrane[7].

The erythrocyte is normally 8μm diameter must be altered to allow the cell to transverse capillaries with 2-3μm. RBCs must be able to undergo repeated passive deformation to fulfill its primary physiological function of oxygen delivery[8]. In addition, the cell must have the capacity to resist fragmentation. These two essential qualities require a membrane that is extremely deformable yet remarkably stable [9]. Membrane stability can be defined as the maximum extent of deformation that membrane undergoes and still completely recover its original shape. Beyond the degree of deformation, the membrane is unable to recover is performed state and it fails[10]. An erythrocyte with normal membrane stability can circulate without fragmenting, while a cell with decreased stability may fragment under normal circulatory stresses[11]. Membrane deformability, on the other hand, determines the extent of membrane deformation that can be induced by a defined level of applied force. A more deformable membrane required less applied force to enable it to pass through tiny capillaries[12].

Therefore, one would predict that membrane deformability and stability woud change if the number or quality of protein-protein association changed. The membrane elastic shear modules of red cell taken from patients with different membrane protein disorders is proportional to the density of spectrin on the inner side of the membrane and this supports the view that spectrin is primarily responsible for shear elasticity[13].

During blood storage a number of intracellular changes occur, such as: decrease of K^+ and increase of Na^+ and Ca^+; decrease of 2,3-diphosphoglycerate, accompanied by an increase of osmotic fragility; decrease of adenosine triphosphate (ATP); accumulation of lactate; changes of the normal discoid shape into speculated cells; decrease of deformability , and decrease post-transfusion survival. Many of the changes are interrelated in more or less well-known way. Most of the metabolic changes are reversible in the circulation within the scope of hours or days[14].

The biological effects of radiation appear either directly when a molecule is ionized and/or excited by the incident radiation on the molecular, cellular level and the biological system as whole. Or indirectly when a molecule reacts with a molecule or the product of a molecule that has undergone events to describe the death of the cell. Since water is a major constituent of all biological materials, water enters into the reaction that is believed to eventually cause the death of the cell after irradiation by formation of free radicals[15].

Materials and Methods

Blood sample collection and storage

Venous blood samples were collected from healthy volunteers (using needle gage 23) anticoagulated with CPDA-1 (Citrate-Phosphate-Dextrose-Adenine-1) in ratio of 2 parts CPDA-1 to 8 parts of blood. Each blood sample was divided into 3 aliquots; one aliquot was processed immediately, where as the other 2 aliquots were stored at 2-4 $^{\circ}$C for 15 and 30 days and processed after the respective storage time.

The fresh or stored anticoagulated blood sample was centrifuged for 4 minutes at 2000 revolution per minute (RPM). The plasma, Buffy coat and other upper most layer of packed RBCs were discarded by gentle aspiration. The resulted packed cells were washed three times by resuspending them with suspending solution containing (in mM) : NaCl 150 , Tris-HCl 10, pH 7.4, recenterfugation, and reasperation of the supernatant and other upper most layer of packed RBCs .The separated red cells were resuspended in the same solution at a haemotocrit (Hct) of 10% or 0.1%.

The source of radiation.

^{60}CO is a Gamma source which decays in probability of 99% by neutron disintegration to the level 2.507 Mega electron Volt (MeV), then the proton de-excites to the lowest stable state by two steppes of de-excitation through emitting two gamma rays cascade of 1.7 and 1.33 MeV state. The life time of the 1.33 MeV state is only 0.7 Pico second (PS) to form stable ^{60}Ni.

Stability measurement

Coaxial cylinder viscometer (Roto Visco RV-11) can establish the reheological properties of fluids over very wide range of defined shear. Results are available in internationally accepted units. The test substance is located in the gap between a rotor and stator. The rotor is driven at a constant speed 486 RPM giving shear rate of $2628S^{-1}$. The whole sensor system was kept at 37^{o}C by water circulator system and all measurements were carried out at this temperature.

Methods of irradiation of RBC suspension

A Sample of 10% Hct red cell suspension was divided into two equal aliquots; the first aliquot was kept without irradiation and served as control. the other aliquot was transferred to plane tubes 10 ml and setup in the tank (phantom) containing bolus grains and covered by a plate of Perspex (0.5 cm thickness), the Perspex represented surface of the phantom, so that the blood sample was located immediately under the Perspex .The tank with bolus grains and Perspex plate may be considered as tissue equivalent. This arrangement was set up under

the window of the therapy unit (^{60}CO, γ -ray emitter) in a limited field size of 12*12 cm at source-surface distance (SSD) of 80 cm and dose rate of 73.2 cGy/min. The samples were irradiated with Gamma-ray of 100 cGy or 1000 cGy from ^{60}CO source of mean energy 1.25 MeV.

The Results

Effect of storage time and radiation dose (1Gy and 10Gy) on the spontaneous haemolysis of RBCs

The absorbance of the non-irradiated RBCs suspension (fresh RBCs) was 0.031+0.001. Exposure of RBCs suspension to radiation dose of 1 Gy and 10Gy was associated with a significant increase in the absorbance of the supernatant of RBC suspension (by 74% and 197% respectively) compared to the absorbance of non-irradiated RBC suspension Fig.(1). In addition, the absorbance of RBC suspension exposed to 10 Gy was also significantly higher (by 70%) relative to the absorbance of RBCs suspension exposed to 1 Gy Fig.(1).

In addition, the absorbance of the supernatant of non-irradiated RBCs suspension stored for 15 days was 0.047+0.002. Exposure of such RBCs suspension to radiation dose of 1 Gy and 10 Gy was associated with a significant increase in the absorbance of the supernatant (by 64% and 115% respectively) compared to the absorbance supernatant of non-irradiated RBC suspension Fig.(2). In addition, the absorbance of the supernatant of RBC suspension exposed to 10 Gy was also significantly higher (by 31%) relative to the absorbance of supernatant of cells exposed to 1 Gy Fig.(2).

Furthermore, the absorbance of the supernatant of non-irradiated RBCs suspension stored for 30 days was 0.064+0.001. Exposure of RBCs suspension to radiation dose of 1 Gy and 10 Gy was associated with a significant increase in the absorbance of the supernatant (by77% and 178% respectively) compared to the absorbance supernatant of non-irradiated RBC suspension Fig.(3). In addition, the absorbance of the supernatant of RBCs suspension exposed to 10 Gy was also significantly higher (by 58%) relative to the absorbance of supernatant of cells exposed to 1 Gy Fig.(3). The absorbance of the supernatant of stored RBCs suspension exposed to 0 Gy , 1Gy and 10 Gy at storage times o day (fresh RBCs) , 15 days and 30 days is illustrated in Fig.(4).

Effect of storage time on the stability of RBCs.

At a fixed shear rate of 2628 S^{-1} and stressing time of 2 minutes, the absorbance of the supertanant of shear stressed fresh cell suspension (day 0) was 0.061+0.005 Fig.(5). Prolongation of the storage time for 15 and 30 days was associated with a significant increase in the absorbance of the supernatant of shear stressed fresh cell suspension (by18% and 30% respectively) compared to the absorbance supernatant of cell suspension at 0 day Fig.(5).

Effect of 1 Gy radiation on the stability of stored RBCs.

At a fixed shear rate of 2628 S^{-1} and stressing time of 2 minutes, the absorbance of the supertanant of irradiated (by 1Gy) shear stressed fresh cell suspension (day 0) was 0.091+0.002 which was not significantly different for cell stored for 15 and 30 days and exposed to 1Gy radiation (0.094 + 0.001 and 0.088 + 0.001 respectively) Fig.(6).

Effect of 10 Gy radiation on the stability of stored RBCs.

At a fixed shear rate of 2628 S^{-1} and stressing time of 2 minutes, the absorbance of the supertanant of irradiated (by 10Gy) shear stressed fresh cell suspension (day 0) was 0.106+0.002 Fig.(7). Storage of RBCs for 15 days was associated with a significant increase in the absorbance of the supernatant of irradiated shear stressed cell suspension by (9.0%) compared to the absorbance supernatant of irradiated shear stressed cell at 0 day. However, prolongation of the storage time for 30 days was associated with a significant decrease in the absorbance of the supernatant of irradiated shear stressed cell suspension (by8.5%) compared to the absorbance of the supernatant of irradiated shear stressed cell suspension at 0 day Fig.(7). In addition, the absorbance of the supernatant of irradiated shear stressed cell suspension at 30 days was also significantly lower (by16%)

relative to the absorbance of the supernatant of irradiated cell suspension at 15 day.

Discussion

In the current research, the membrane stability was used to detect indirectly the changes in the membrane protein of RBCs stored at different times under blood bank storage conditions. Physical factor such as radiation was well known factor reported to change the RBC membrane proteins.

Radiation-induced spontaneous haemolysis.

In the present study, the effect of radiation dose on the spontaneous haemolysis of RBCs was studied on fresh and stored blood. Spontaneous haemolysis was found to increase as the radiation dose increased. It is possible to suggest that the increase in the percentage of spontaneously haemolyzed RBC was due to the damage of membrane protein as a result of the free radicals, which attack membrane proteins. This suggestion was supported by the finding that the γ-rays induced free radical generation as it pass through water [15]. The increase of the spontaneously haemolyzed RBCs upon radiation as RBC stored for prolonged period of time was possibly due to impairment of antioxiative systems of RBC. This suggestion was supported by the finding that the superoxide dismutase activity (antioxidative enzyme) was reduced with storage of blood [16].

Radiation effect on stability of RBCs

Exposure of fresh RBC to a dose of 1Gy-radiation did not affect the stability of RBC membrane. In contrast, exposure of fresh RBC to a dose of 10Gy-radiation was associated with a significant decrease in the stability of RBC membrane upon stress. It is possible to suggest that the lack effect of 1Gy-radiation and the positive effect of 10Gy-radiation on the stability of RBC membrane were due to less free radical generation at the former radiation dose relative to the latter radiation dose. This possibly was supported by the finding that the amount of free radicals generated by the passage of γ-rays was proportional to the dose of the radiation [17]. In addition, exposure of RBCs to a dose of 1Gy-radiation did not affect the stability of RBC membrane which was stored for up to 30 days. This again, may be explained that the free radical generation by this radiation dose was small enough to be scavenging red by the available antioxidative systems. This suggestion was supported by the finding that some antioxidative enzyme systems, was still intact in stored blood such as catalyses and gultathione peroxidase [8]. The decrease in the stability of RBC membrane stored for 15 days upon 10Gy radiation was again due to the production of free radicals which may overwhelm the available antioxidative enzyme systems. However, the reduction of haemolysis upon stress of RBC stored for 30 days which were exposed to 10 Gy-radiation may be due to failure of Hb release by the ruptured RBCs. This failure of Hb release was possibly due to direct Hb-membrane bonding. This suggestion was supported by the finding that the antioxidative enzyme systems upon stored blood was less efficient to scavenge red the large amount of free radicals generation and consequently may cause damage to both Hb and membrane protein simultaneously. Therefore, a change of interaction between the Hb and membrane proteins become evident. In fact, Morizawa et al, 1992, were able to show that treatment of RBC with H2O2 (free radical generation agent) led to formation of Hb-Spectrin complex as a result of change in both of them and consequent intermolecular binding [18].

Conclusions

1. Membrane protein changes during storage of blood decreased the stability of RBC.
2. Exposure of RBCs to a dose of 1Gy-radiation did not affect the stability of fresh or stored RBCs.
3. Exposure of RBCs to a dose of 10Gy-radiation decreased the stability of fresh or stored RBCs.

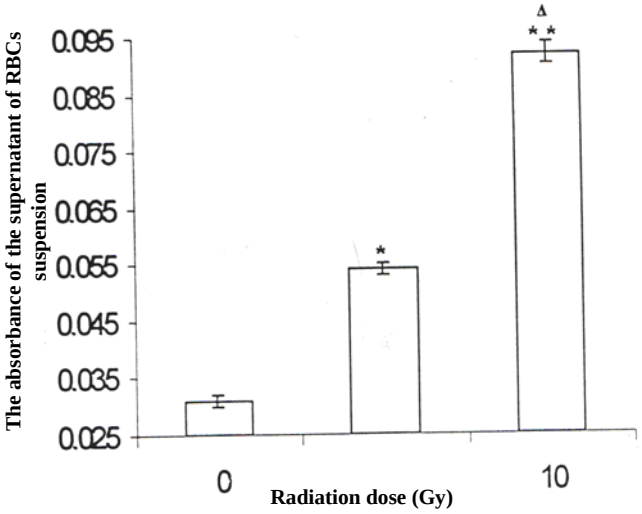

*Fig.(1): The absorbance of the supernatant of fresh RBCs suspension (day 0) exposed to radiation dose to 1 Gy and 10 Gy. Each value represents mean ± SEM of six experiments. * = P< 0.001, ** = P< 0.001 in comparison with supernatant of non-irradiated RBC suspension (0 Gy). Δ =P< 0.001 relative to 1 Gy radiation dose.*

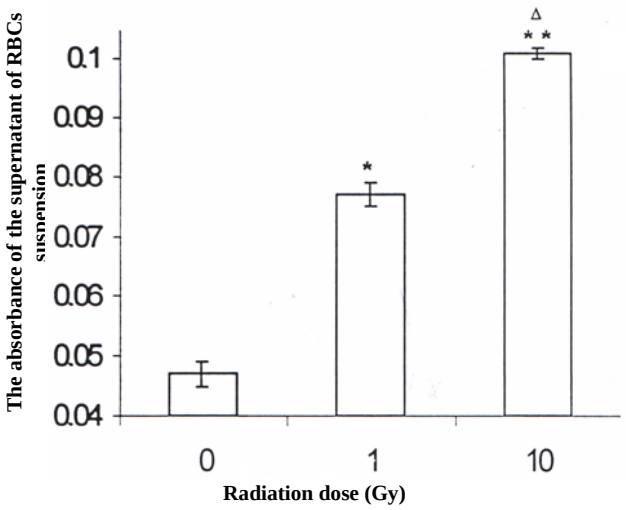

*Fig.(2) : The absorbance of the supernatant of stored RBCs suspension (day 15) exposed to radiation dose to 1 Gy and 10 Gy. Each value represents mean ± SEM of six experiments. * = P< 0.001, ** = P< 0.001 in comparison with supernatant of non-irradiated RBC suspension (0 Gy). Δ =P< 0.001 relative to 1 Gy radiation dose.*

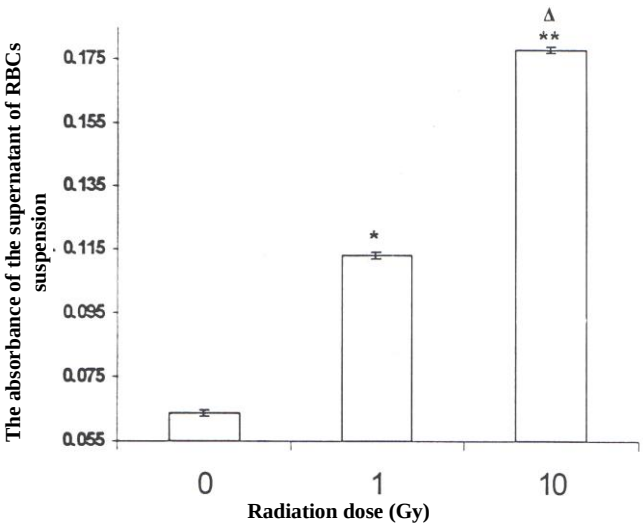

*Fig.(3): The absorbance of the supernatant of stored RBCs suspension (day 30) exposed to radiation dose to 1 Gy and 10 Gy. Each value represents mean ± SEM of six experiments. * = P< 0.001, ** = P< 0.001 in comparison with supernatant of non-irradiated RBC suspension (0 Gy). Δ =P< 0.001 relative to 1 Gy radiation dose.*

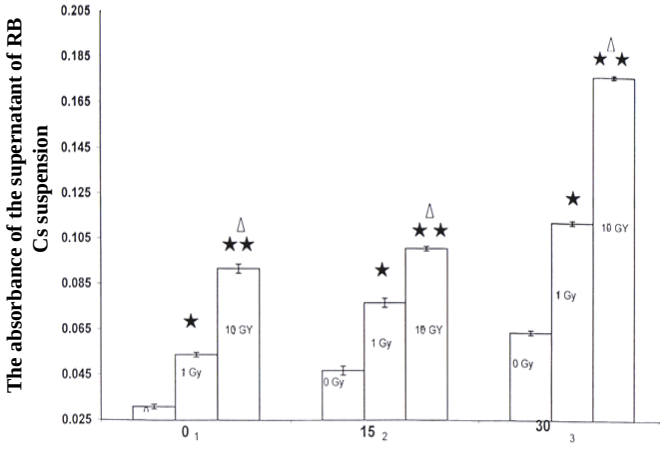

Fig. (4): Accumulation figures 1,2 and 3.

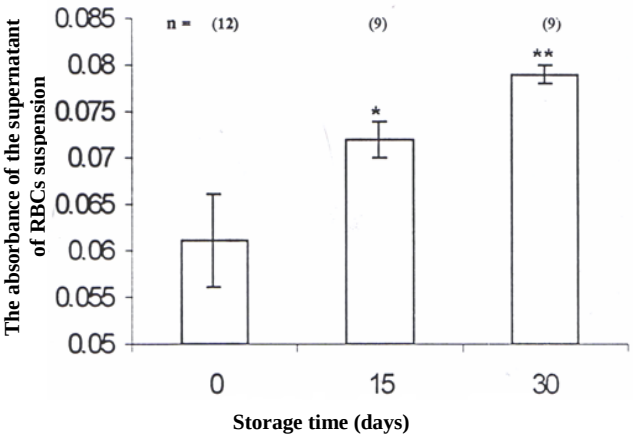

Fig. (5): The absorbance of the supernatant of stressed RBCs suspension stored at different times under blood bank storage conditions. Each value represents mean ± SEM of the number of observations (n) shown between brackets. * = P< 0.001, ** = P< 0.001 in comparison with that of fresh cells (day 0).

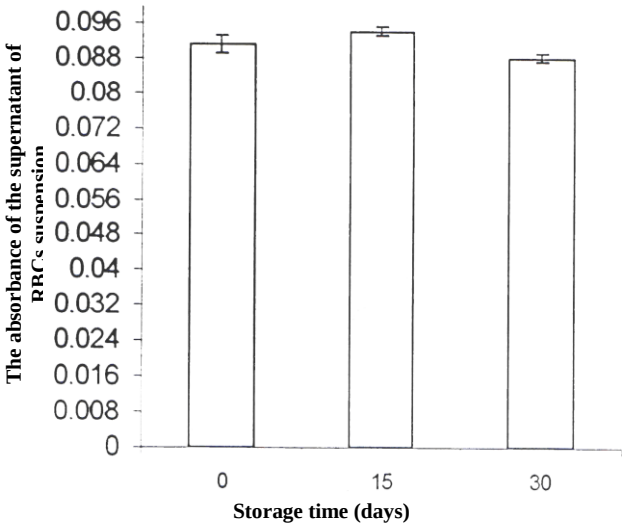

Fig. (6): The absorbance of the supernatant of stressed RBCs suspension stored at different times under blood bank storage conditions exposed to 1 Gy radiation dose. Each value represents mean ± SEM of 6 experiments

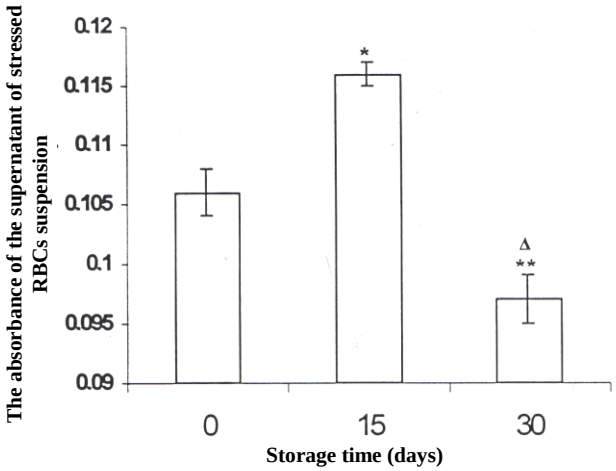

Fig. (7): The absorbance of the supernatant of stressed RBCs suspension stored at different times under blood bank storage conditions exposed to 1 Gy radiation dose. Each value represents mean ± SEM of 6 experiments. * = P< 0.05, ** = P< 0.02 in comparison with that of fresh cells suspension (day 0). Δ =P< 0.01 relative to 15 days.

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الخلاصة

لقد وجد ان خلايا الدم الحمراء المخزونة تعاني من عدة تغيرات في بروتينات الغشاء. في دراستنا الحالية تم اختيار استجابة كريات الدم الحمراء المخزونة بدلالة استقرارية غشاء كريات الدم الحمراء عند تعرضها لا شعة كاما. اظهرت كريات الدم الحمراء المخزونة لمدة 15 و 30 يوما نقصانا في استقراريتها مقارنة باليوم الاول, بالاضافة ا لى ذلك لم يؤثر تعريضها الى جرعة اشعاعية (1 غري) من اشعة كاما على استقرارية كريات الدم الحمراء الحديثة والمخزونة. بينما عند تعريض كريات الدم الحمراء المخزونة لمدة 15 يوم لجرعة اشعاعية (10 غري) من اشعة كاما وجد ان استقرارية هذة الخلايا تقل مقارنة باستقراريتها في اليوم الاول اما بالنسبة للخلايا المخزونة لمدة 30 يوم فقد لوحظ زيادة ظاهرية في استقراريتها عند تعرضها الى (10 غري) من اشعة كاما مقارنة باستقرارية كريات الدم الحمراء الحديثة والمخزونة لمدة 15 يوم.