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Electrical behavior of stored erythrocytes after exposure to gamma radiation and the role of α-lipoic acid as radioprotector

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ABSTRACT

The effects of γ rays (25, 50 and 100 Gy) on stored erythrocytes were studied by measuring their dielectric properties and observing their morphology under scanning electron microscopy. Alpha lipoic acid (a potent natural antioxidant) was introduced prior to irradiation for radioprotection. It can be concluded that the dose level of 25 Gy can be considered a safe sterile dose; however, irradiation doses of 50 and 100 Gy should be applied with the addition of α-acid to preserve the cell viability.

1. Introduction

Irradiation of donor's blood with γ-rays has been adopted at blood banks as a strategy to avoid transfusion-associated graft-vs-host disease (TA-GVHD) (Seghatchian and Ala, 1995). TA-GVHD occurs when severely immuno-deficient or immuno-depressed patients receive blood containing donor's immunologically functional lymphocytes. The donor's lymphocytes could develop an immune response against host cells. Irradiation of blood with γ-rays is known to inactivate the T-lymphocytes that trigger TA-GVHD and has been described as a tool to prevent this illness (Anderson, 2003). γ-rays damage the T-lymphocytes and arrest responses to allogeneic cells. Thus, these lymphocytes are unable to proliferate in the host and therefore cannot mediate TA-GVHS. The use of γ-rays is limited by its damaging effects on erythrocytes and platelets. The dose of 25 Gy is recommended for irradiation of blood bags, as it is considered safe for erythrocytes (Jacob, 1998). However, some authors reported that this dose is not enough to inhibit the activity of T-cells completely (Fagiolo and Toriani-Terenzi, 2002), and the debate around the determination of the suitable doses needs further investigation.

Blood banking procedures focus on the damage to erythrocyte membrane, which could possibly impair their flow properties, namely, their deformability, aggregability, and adherence to endothelial cells and thus possibly introducing a circulatory risk to recipients (Relevy et al., 2008). Several studies have been adopted to study the effects of γ-rays on erythrocytes. Many parameters have been chosen. For example, exposure to γ rays (25 Gy) significantly increased the mean corpuscular volume (MCV) and lipid peroxidation (Kim et al., 2008), and increase the permeability of erythrocyte membrane to monovalent and divalent cations and would change ion homeostasis and cell function (Moreira et al., 2008). Exposure to 35 Gy γ-rays resulted in a decrease of erythrocytes deformability, which can be attributed mainly to dehydration due to potassium loss (Cicha et al., 2000). A critical examination of the literature and experimental results was carried out by Reverberi et al. (2007) who concluded that the erythrocyte deformability is the only parameter related to viability which reflects sufficient precocious and important changes. Deformability, as other erythrocytes' properties, depends to a great extent on both mechanical and electrical properties of the cell membrane. It is found that the decrease in surface charge of erythrocytes leads to the decrease in the deformation and orientation indices as well as the increase in blood viscosity (Wen et al., 2000).

Erythrocytes, like all body cells, possess electrical properties which arise from their membrane structure and intra- and extracellular contents. Dealing with radiation effects at the electrical level may have some benefit in defining the effects of radiation as well as in the proposal of possible treatments. Measurement of the cell dielectric properties could reveal important information about the cell's physiology, in particular the properties of the

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membrane and the cell interior. Several studies investigated the sensitivity of the dielectric parameters to the physiological and structural states of erythrocytes, which resulted in the introduction of fitted equations to obtain viscosity (Pop et al., 2003) and ATP, K, Na and Cl concentration (Sezdi et al., 2006) from dielectric measurements.

Radiation-induced oxidative damage in erythrocytes is considered as a result of the disturbed balance between its protective systems and the level of toxic oxygen products. This radiation damage could be counteracted by the addition of pharmacological antioxidants such as ceruloplasmin, transferrin, edaravone, and melatonin. In addition, several provitamins and vitamins have been found to be potential radioprotectors (Manda and Bhatia, 2003). Among the extensively considered antioxidants is α-lipoic acid (ALA). It is unique among antioxidant molecules because it retains protective functions in both its reduced and oxidized forms. It has been found that exogenously supplied α-lipoic acid has antioxidant properties and is effective in preventing or minimizing the damage caused by reactive oxygen species (ROS). Its properties as an antioxidant have recently been reviewed (Bilska and Wlodek, 2005). ALA, or its reduced form, dihydrolipoic acid (DHLA), quenches a number of oxygen free radical species in both lipid and aqueous phases, chelates transition metals, and prevents membrane lipid peroxidation and protein damage. The aim of the present work is to ensure that the currently used γ-rays dose (25 Gy) in the sterilization of blood bags before being transferred to patients is a safe dose, and to exploit the feasibility of elevating this dose up to 100 Gy to ensure the elimination of T-lymphocyte cells through the addition of α-lipoic acid. The protective properties of this anti-oxidant are evaluated at different γ-rays doses (25, 50 and 100 Gy) through studying the electrical properties of stored erythrocytes and scanning electron micrography (SEM).

2. Materials and methods

2.1. Chemicals

D1-α lipoic acid (M.wt. 206) was purchased from Fluka Biochemica, sulfoxide dimethylsulfoxide (DMSO) (M.wt. 78.13) was purchased from Riedel-Dehaen.

2.2. Blood samples

Ten bags of packed erythrocytes were obtained from the blood bank of the Egyptian Red Crescent, after they have been checked for viruses. The donors were males raging in age from 25 to 35 years.

2.3. Preparation of erythrocytes samples

Erythrocytes were washed by phosphate-buffer saline (PBS) and centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatant was removed to obtain the packed cells. Separated erythrocytes were suspended to 20% hematocrit in PBS containing 2 mM glucose 5% (prepared for intravenous infusion pyrogen free). Lipoic acid, dissolved in DMSO, was added to erythrocytes suspensions in concentration of 123 μmol/l. The samples were incubated in water bath at 35 °C for 30 min. After incubation the samples were centrifuged at 3000 rpm for 10 min to obtain packed erythrocytes.

2.4. Gamma irradiation

The irradiation process was carried out at the National Center for Radiation Research and Technology (NCCRT), Atomic Energy Authority, Cairo, Egypt. The γ-rays doses were 25, 50 and 100 Gy. The dose 25 Gy was obtained from a Cesium-137 source with a dose rate of 0.845 rad/s, while 50 and 100 Gy doses were obtained from Cobalt-60 source (dose rate was 0.56170 kGy/h). Dose calibrations of the gamma sources were performed by the Egyptian high-dose reference laboratory. Blood samples were maintained in ice bath during the period of irradiation. All measured parameters were carried out immediately after irradiation.

2.5. Morphological analysis

The morphology of fresh and stored erythrocytes was studied using scanning electron microscope (SEM) according to Ross et al. (2007) as follows:

Three drops of erythrocyte suspension were added directly to 5 ml of 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Fixation was allowed to proceed for at least 24 h before processing. The cells were washed twice in cacodylate buffer, dehydrated with two washes in 70% ethanol, washed twice in 95% ethanol, twice in absolute ethanol and twice in acetone. One drop of the cell suspension was applied to an acetone-washed cover slip and allowed to dry. The cover slip was fixed to an aluminum stub using a colloidal silver adhesive and gold coated using a sputter coater (SPI). Electron microscopy was carried out using a Jeol Model JSM-5400 scanning electron microscope at the National Center for Radiation Research and Technology (NCRRT). The cells were examined microscopically and classified according to their shapes.

2.6. Dielectric measurements

The dielectric measurements were performed using LCR meter HIOKI 3531, manufactured in Japan, in the frequency range 40 kHz to 5 MHz. The measured parameters are admittance (Y), phase angle (θ), reactance (X) and susceptance (B) which allows capacitance (C), conductance (G) and impedance (Z) analysis using the appropriate relations. All the calculations were carried out by means of the LCR meter software. The measuring cell is a parallel plate conductivity cell with platinum electrodes of 4 cm^2 area separated by a distance of 2 cm. To reduce the electrode polarization during the measurements, the following steps were carried out. The electrodes were platinum coated with platinum black layer known to have the lowest possible impedance (Schwan, 1983; Iwamoto and Kumagai, 1998). The cell size is made as large as possible in order to decrease the electrode impedance (Grosse and Tirado, 2002). The sample volume in this study was 8 cm^3. Measurement cell connection was made from coaxial shielded silver wire, to eliminate stray capacitance. The cell was calibrated using liquids with known permittivity values (acetone, methanol and ethanol). The erythrocytes suspension (Hct 3%) was suspended in buffered saline (pH 7.4 and conductivity 0.627 S/m). The samples were incubated in water bath at 37 °C during the measurements.

The relative permittivity ε' can be calculated as follows:

$$\varepsilon' = \frac{C}{\varepsilon_0}\frac{d}{A} \quad (1)$$

where A is the surface area of the electrodes, d the distance between the two electrodes, and ε_0 the vacuum permittivity.