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PRELIMINARY STUDY OF ALTERATIONS IN HUMAN RED BLOOD CELLS BY IRRADIATION WITH HIGH ENERGY PHOTONS

Bibiana Riquelme[1,2]*, Ezequiel Estrada[3], Horacio Castellini[3], Andrea Acosta[4], Alejandro Chinelatto[4], Ivan Tac[4], Javier Borraz[4], Liliana Di Tullio[4], Mariel E Galassi[1,3]

[1]Grupo de Física Biomédica, IFIR (CONICET-UNR); [2]FCByF (UNR); [3]FCEIA (UNR); [4]CRH (Santa Fe)
*briquel@fbioyf.unr.edu.ar, riquelme@ifir-conicet.gov.ar

INTRODUCTION

Transfusion-associated graft-versus-host disease can be prevented by treating cellular blood products with gamma irradiation. A wide range of gamma irradiation dose levels are used in routine practice, but gamma irradiation dose of 25 Gy may be required to completely inactivate T cells in Red Blood Cells (RBC) units (Pelszynski, M. et al., 1994). This process decreases the survival of the RBC transfused, so it is crucial to understand the alterations caused by gamma irradiation to the erythrocyte membrane. In previous works, the biochemical and hematological effects of gamma irradiation at different storage periods were studied. It was observed that irradiation of the erythrocytes increases red cells hemolysis and leakage of intracellular potassium (Adams, F. et al., 2015; Yousuf, R. et al., 2018). The mechanisms through which irradiation causes the loss of RBC viability could be related to the primary effects of radiation. Gamma and X-ray Ionizing radiation cause indirect damage through the reactive oxygen species generated by water radiolysis (Anand, A.J. et al., 1997). The reduced deformability of RBC after irradiation could be related to the interaction of the oxygen-derived radicals with the membranes, affecting their mechanical properties and leading to deformability impairment (Kim, Y.-K. et al., 2008).

In a recent work (AlZahrani K. et al., 2017), nanoestructural changes in the RBC membrane at different doses of gamma irradiation were observed using atomic force microscopy. The images shown that the roughness of the cell membrane increased dramatically with increasing doses, affecting their biophysical properties. However, more research is needed to understand the effects of gamma irradiation on the mechanical and adhesion properties of RBC. For this reason, in the present work we set out to measure the mechanical and aggregation parameters of human red blood cells exposed to gamma photons in different doses in order to determine the possible alterations due to radiation.

MATERIALS AND METHODS

Red blood cells (RBC): Fresh blood sample was collected from healthy donor by vein puncture, in sterile vials containing EDTA as anticoagulant. Samples were processed within 4 hours of extraction, as recommended in Baskurt et al. (2007).

Irradiation: Blood samples were irradiated in the "Centro Regional de Hemoterapia de Santa Fe" using Biobeam GM 8000 (Gamma-Service Medical GmbH) with a source of Cesio-137 (emission peak at 661,7 keV) at 1.5 Gy, 5 Gy, 10 Gy, 15 Gy and 25 Gy. The irradiation was carry out in a specific device for blood components (see Figures 1 and 2).

Figure 1. Experimental device and Biobeam facility.

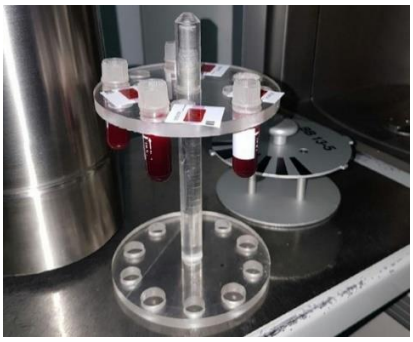

Figure 2. PMMA sample holder.

Viscoelastic parameters of red blood cells: Data were obtained using the Erythrocyte Rheometer (Albea et al., 2013), a new instrument developed in our laboratory that gives stationary and dynamic viscoelastic parameters of red blood cells (Riquelme et al., 1998, 1999 and 2006): erythrocyte deformability index (ID); elastic modulus of erythrocyte (μ) and surface viscosity of erythrocyte membrane (η). To carry out these measurements by quintuplicate, 100 μL of each blood sample was poured into 4.5 mL of a solution of polyvinylpyrrolidone (Sigma PVP360) at 5% (w/v) in PBS.

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Optical chip erythrocyte aggregometer: Changes induced by the irradiation on erythrocyte aggregation were evaluated by an optical chip, based on the analysis of laser transmission through a blood sample recorded in real time (Toderi et al., 2015). Triplicate determinations on blood samples were performed for controls and irradiated RBCs.

RESULTS AND DISCUSSION

Graphics of light intensity as a function of time (Figure 3) were obtained and the time required to reach half of the total transmitted light intensity (t1/2) was calculated for each blood sample.

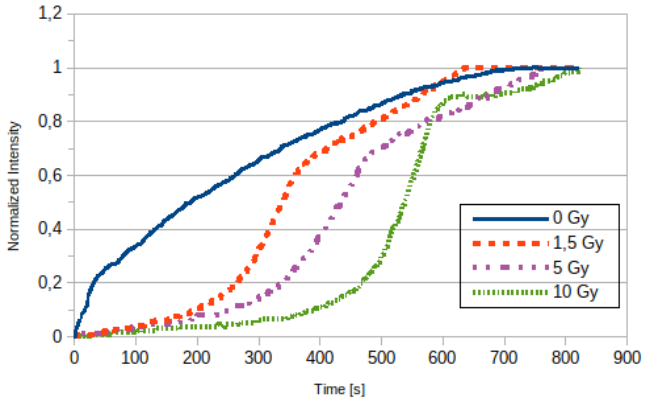

Figure 3. Kinetics aggregations curves for RBC irradiated at different doses.

A sigmoidal fitting of the RBC aggregation curves of Figure 3 was made for each doses. The time to reach half of the total aggregation ($t_{1/2}$) was calculated (Table 1) showing a significantly increase with the irradiation dose.

Table 1. Time to reach half of the total erythrocyte aggregation.

Sample	$t_{1/2}$ [s]	SD [s]
Control	204	10
1.5 Gy	367	18
5 Gy	463	23
10 Gy	553	28

Table 2 shows the viscoelastic parameters of RBCs obtained with the Erythrocyte Rheometer.

Table 2. Viscoelastic parameters of red blood cells.

Sample	ID		μ [10^{-6} N/m]		η [10^{-7} N.s/m]	
	Mean	SD	Mean	SD	Mean	SD
Control	0.61	0.04	5.5	0.1	2.84	0.05
10 Gy	0.40*	0.01	5.9	0.1	2.67*	0.08
25 Gy	0.35*	0.01	5.5	0.1	2.07**	0.02

* $p < 0.05$; ** $p < 10^{-4}$

Results have not shown alteration in the erythrocyte elastic modulus (μ) but deformability index (ID) significantly decrease with the irradiation doses indicating a rigidizing of the erythrocyte membrane, which is related with a significantly decrease in the surface viscosity (η) of membrane.

CONCLUSION

Obtained results suggest an important alteration in the lipids and glucoporoteins of erythrocyte membrane by effect of gamma irradiation that is more pronounced at the higher doses. The present study is very important to understand the alterations produced by gamma radiation on RBC and how it can affect the survival of the transfused red blood cells. The continuation of these investigations could allow the optimization of the irradiation protocols in the blood banks in order to prolong the useful times of the stored blood.

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