The effect of timing of gamma-irradiation on hemolysis and potassium release in leukoreduced red cell concentrates stored in SAGM

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While irradiation of red cell concentrates (RCC) prevents graft-versus-host disease in susceptible transfusion recipients, it also damages red blood cells (RBC). To understand the ability of irradiation regulations to prevent transfusion of inferior units, we irradiated 980 RCC in saline-adenine-glucose-mannitol (SAGM) using various combinations of pre-irradiation age and post-irradiation storage times, and measured hemolysis and extracellular potassium levels. We observed unacceptably high hemolysis (≥0.8%) in some RCC and elevated extracellular potassium levels in all gamma-irradiated RCC. This suggests that more restrictive storage times should be considered for RCC in SAGM.

Key words: blood components, component production, quality control.

Introduction

Gamma-irradiation of red cell concentrates (RCC) reduces the risk of graft-versus-host disease in immunocompromised transfusion recipients [1]. However, gamma-irradiation also produces reactive oxygen species (ROS) that cause lipid peroxidation and membrane damage. This reduces RBC function and viability [2], which may diminish post-transfusion recovery and lead to adverse post-transfusion outcomes.

Current AABB gamma-irradiation guidelines, which are also used in Canada, indicate that irradiation may be performed at any time during RCC storage. Irradiated RCC may be stored up to a maximum of 28 days post-irradiation. Based in part on a recent study [2], the latest update of the Council of Europe guide (17th edition) shortened the allowable post-irradiation storage time for irradiated RCC from 28 days to 14 days. [3] The revised guideline states ‘Red cell components may be irradiated up to 28 days after collection. Irradiated cells must be transfused as soon as possible, but no later than 14 days after irradiation and, in any case, no later than 28 days after collection.’ The British Committee for Standards in Haematology (BCSH) has even more restrictive standards, stating ‘Red cells may be irradiated at any time up to 14 days after collection, and thereafter may be stored for a further 14 days.’ [4] We investigated the impact of length of RCC storage before and after gamma-irradiation on hemolysis and extracellular potassium levels.

Methods

Leukoreduced RCC (n = 896) in saline-adenine-glucose-mannitol (SAGM) were manufactured from whole blood collected into citrate-phosphate-dextrose. Whole blood was leukoreduced at 4–8°C, prior to component production according to Canadian Blood Services standard operating procedures. RCC were stored in PVC-DEHP containers (Fenwal, Lake Zurich, IL, USA).

Red cell concentrates were gamma-irradiated (25 Gy) 8–40 days after collection and subsequently stored for 1–28 days without exceeding total storage time of 42 days [1]. Hemolysis, measured once for each RCC, was calculated from hematocrit and total hemoglobin (Hb) measurements made by a hematology analyzer.
were considered significant at \( P < 0.05 \).

A separate set (\( n = 84 \)) of similarly produced RCC were gamma-irradiated 7, 10, 14, 21, 35, or 40 days post-collection. Testing began 2 days post-irradiation, with daily sampling for the first week (excluding weekends), then weekly until either 28 days post-irradiation or the 42 day expiry of the RCC, whichever occurred first. Extracellular potassium was measured by indirect potentiometry (DXC 800; Beckman Coulter Inc., Fullerton, CA, USA).

Multiple linear regression analysis was performed on log-transformed hemolysis data using Minitab 16 (Minitab Inc., State College, PA, USA). Explanatory variables included length of storage before and after irradiation (\( r^2 = 0.53 \)) indicated the length of storage before or after irradiation predicted hemolysis (\( P < 0.001 \)) as did male gender (\( P = 0.015 \)), and greater donor age (\( P < 0.001 \)). Storage time after irradiation was the most predictive explanatory variable for potassium. RCC irradiated in their fourth week of storage and subsequently stored for 15–21 days exhibited the highest level of hemolysis (mean hemolysis = 0.66 ± 0.44%; 18 units >0.8% ; \( n = 57 \)). Hemolysis was less pronounced in RCC irradiated after 4 weeks due to limited storage time after irradiation.

Extracellular potassium levels increased rapidly following gamma-irradiation, reaching levels comparable to those of non-irradiated 42-day RCC within 3 days of irradiation, regardless of unit age when irradiated (Fig. 2). According to multiple linear regression analysis (\( r^2 = 0.83 \)), explanatory variables predictive of potassium concentration included length of storage before and after irradiation (\( P < 0.001 \)), male gender (\( P < 0.001 \)), and greater donor age (\( P < 0.05 \)).

**Discussion**

We observed enhanced hemolysis in RCC irradiated later in storage compared to those irradiated earlier, consistent with the reduced antioxidant protective capacity in older RBC [5]. This analysis indicates that adherence to North American RCC irradiation guidelines can lead to poor *in vitro* quality for RBC in SAGM. Units with non-conforming hemolysis were rarely seen using either Council of Europe guidelines or BCSH standards. While there is some indication that storage in different additive solutions (AS) can result in different hemolysis levels [6], evidence is lacking regarding the degree of difference in hemolysis that may exist after gamma-irradiation, if any. Studies investigating hemolysis rates of gamma-irradiated RBC in other AS could be considered for regions with less restrictive gamma-irradiation guidelines.

Unlike non-irradiated RCC, which linearly increase extracellular potassium levels over storage time [7], an initial sharp increase in potassium was detected following gamma-irradiation. This further suggests that gamma-
irradiation compromises RBC membrane integrity, possibly directly affecting Na⁺/K⁺-ATPase function [8]. Such a dramatic increase in potassium levels may escalate the risk of post-transfusion hyperkalemia and subsequent cardiac complications in neonates or patients requiring massive transfusions [7].

The data reported here, which represent the largest series in the literature, demonstrate that timing of gamma-irradiation has a significant impact on in vitro RBC quality. With notably increased hemolysis and extracellular potassium levels in RCC irradiated late in storage, gamma-irradiation guidelines should be examined and potentially revised to avoid transfusion of inferior quality RCC.

Conflict of interest

The authors declare no conflict of interests.

Author contribution

DVD conceived the study and edited the manuscript. KS, DC, and EL designed the detailed study protocol for the large scale irradiation component, executed the study and performed the data analysis; DC wrote the initial manuscript. JPA designed the study protocol for the smaller hemolysis/potassium study which was executed and analyzed by ALH, TRT and JDRK.

References

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