Irradiation Effect On Aging Red Blood Cells*

ELAINE K. JETER, M.D., RICHARD H. GADSDEN, PH.D.,
and JOHN C. CATE, IV, M.D.

Department of Pathology/Laboratory Medicine,
Medical University of South Carolina,
Charleston, SC 29425-0701

ABSTRACT

Irradiation of stored red blood cells (RBC) is increasingly utilized for patients who are immunosuppressed or on chemotherapeutic regimens. With the growing demand for irradiated cellular blood products, there has been an increasing need for transfusion services to store previously irradiated blood until needed for transfusion. The effect of irradiation on aging stored RBC has not been studied to date. Five units each of group A, RBC collected in CPD-Adsol (AS-1) with a prior shelf-life of 10, 20, 30, and 40 days, respectively, were divided equally utilizing a sterile docking device and stored at 1 to 6°C. Baseline samples from each bag were obtained for the measurement of extracellular potassium (K+), plasma free hemoglobin (PFH), total lactate dehydrogenase (LD), and erythrocyte 2,3-DPG activity. One of each pair received 2,000 rads of gamma irradiation. Samples were obtained at 3 and 7 days post-irradiation, and multiples of 7 days until expiration. All irradiated units reached a state of K+ equilibrium at 60 to 70 mmol per L irrespective of the length of previous storage with an inverse relationship of RBC age at irradiation and the time required to reach the state of equilibrium. Increased K+ leakage from irradiated aging RBC suggests the need for including in vivo studies of cell survival to establish a post-irradiation storage life. Length of storage prior to irradiation had no effect on PFH, LD activity, and 2,3-diphosphoglycerate (2,3-DPG) activity compared to paired controls.

Introduction

Recipients of blood from first degree relatives, neonates receiving exchange transfusions, and patients who are immunosuppressed or on intensive chemotherapeutic regimens are candidates for irradiated blood or cellular blood products. With the growing demand for irradiated blood products, there has been an increasing need for transfusion services, which often are not equipped with blood irradiators or with access to therapeutic irradiating devices, to store previously irradiated blood until needed for transfu-
IRRADIATION EFFECT ON AGING RED BLOOD CELLS

In many cases, this irradiated blood has been stored for some period of time by a blood center or transfusion service prior to irradiation. It has been shown that RBC, irradiated immediately following collection and subsequently stored, have significantly increased potassium (K⁺) concentrations compared to paired controls throughout their shelf-life, and that irradiated RBC CPDA-1 anticoagulant have significantly greater concentrations of K⁺ at comparable periods of post-irradiation storage than irradiated RBC anticoagulated with AS-1 (RBC AS-1). To our knowledge, one report of whole blood and RBC stored for 21 days (CPD anticoagulant) prior to 5,000 rads of irradiation had significantly different plasma K⁺ levels compared to non-paired controls. Red cell K⁺ loss or extracellular K⁺ gain in previously stored, then irradiated RBC that are subsequently returned to storage has not been reported.

Data are presented on the in vitro post-irradiation effects of 2,000 rads on RBC AS-1 that have been previously stored for 10, 20, 30, and 40 days, respectively, prior to irradiation. Baseline samples were obtained from the paired control and irradiated units immediately prior to irradiation; subsequent samples were taken at designated intervals following return of units to routine storage until expiration (42 days).

Materials and Methods

Five (5) units each of group A, RBC AS-1 with a shelf-life of 10, 20, 30, and 40 days, respectively, were provided by the Carolina Lowcountry American Red Cross (ARC) for this study. The hematocrit was approximately 60 percent in all units. Collection, preparation, storage, and transportation protocols followed standard ARC operating procedures.

Contents of each of the 20 units were equally divided between the parent bag (controls) and a transfer bag (irradiated) via a sterile docking device; sterile sample site couplers were aseptically adapted to each bag. Baseline sampling from each bag was immediately performed under a laminar flow hood. All units were stored at 1 to 6°C until expiration. Each of the complimentary transfer bags was irradiated with 2,000 rads of gamma irradiation. Additional samples were obtained on post-irradiation days 3, 7, and weekly until expiration; sampling intervals for the 30-day and 40-day units were extended beyond the normal expiration date for sufficient data points to demonstrate trends. Cultures were obtained on the seventh day following irradiation and at expiration. All cultures showed no growth after seven days of incubation.

After 30 gentle inversions, 6.5 mL of resuspended red blood cells were collected in 10 mL tubes adapted with 16 gauge needles, and separated into 5.6 mL and 1.0 mL aliquots. The supernatant, collected from the 5.6 mL aliquot, was assayed for potassium (K⁺), total lactate dehydrogenase (LD) activity and plasma free hemoglobin (PFH). Three mL of eight percent trichloroacetic acid were added to the one mL aliquot, incubated at room temperature for 10 minutes and centrifuged at 3,200 rpm for 10 minutes; the protein free supernatant used for 2,3-DPG determination was immediately frozen at −70°C and subsequently assayed in batch analysis.

Supernatant K⁺ determination was made by ion specific electrode. Elevated K⁺ levels were reanalyzed using the urine mode (analytical range: 2.0 to 300.0 mmol per L). Supernatant PFH levels were determined by differential spectrophotometric analysis calculated at

---

* Gammacell 1,000, Atomic Energy of Canada Limited, Radiochemical Co., Kanata, Ontario, Canada.
† Astra-8, Beckman Instruments, Brea, CA 92621.
Figure 1. Mean K⁺ (mmol per L) paired control (CTRL) and irradiated (IRRAD) RBC AS-1, equally divided and irradiated after 10, 20, 30, and 40 days of storage, respectively. Irrespective of the previous storage period, K⁺ increased in all irradiated units to a maximum of 60 to 70 mmol per L; the older units attained this level in significantly less time compared to the fresher units.

Serial mean K⁺ concentrations for paired control and irradiated RBC stored for 10, 20, 30, and 40 days, respectively, prior to irradiation are shown in figure 1. The mean baseline K⁺ at day 10 was 19.4 ± 2.3 mmol per L. On the third and seventh post-irradiation day, the K⁺ values in the irradiated units were 1.6 and 1.7 fold greater than the respective paired control units. Although extracellular K⁺ in the irradiated units continued to rise and were significantly greater than controls at subsequent testing intervals, the ratio of extracellular K⁺ in the irradiated units slowly decreased during storage to a steady state at 1.4 fold greater than the control units. At expiration, the mean K⁺ concentration was 49.0 ± 5.0 mmol per L (CTRL) and 68.2 ± 7.6 mmol per L (IRRAD), respectively.

Baseline mean K⁺ in the units previously stored 20 days prior to irradiation was 29.4 ± 3.8 mmol per L. On the third and seventh post-irradiation day, K⁺ in the irradiated units was 1.6 and 1.8 fold greater than respective control units. At expiration, K⁺ was 1.7 fold greater than the controls; mean K⁺ was 40.4 ± 5.7 mmol per L (CTRL) and 70.8 ± 9.5 mmol per L (IRRAD), respectively.

Units previously stored for 30 days prior to irradiation had baseline mean K⁺ concentration of 38.2 ± 5.5 mmol per L. The K⁺ in the irradiated units was 1.2 fold greater than controls at all desig-
nated testing intervals. At expiration, the mean K⁺ concentrations were 50.8 ± 6.4 mmol per L (CTRL) and 63.6 ± 6.5 mmol per L (IRRAD).

The baseline mean K⁺ concentration for units previously stored for 40 days prior to irradiation was 47.0 ± 3.0 mmol per L. On the third post-irradiation day, K⁺ was 1.2 fold greater than the control units; mean K⁺ was 50.0 ± 4.0 mmol per L (CTRL) and 62.8 ± 2.8 mmol per L (IRRAD), respectively.

Concentrations of PFH, an indicator of hemolysis, in control and irradiated samples are presented in figure 2. Plasma free hemoglobin increased over time in all samples. Baseline mean PFH concentrations at 10, 20, and 30 days of prior storage were 47.2 mg per dL, 62.5 mg per dL, and 90.5 mg per dL, respectively. Baseline PFH in the 40 day control and irradiated units was 175 mg per dL and 235 mg per dL, respectively. Irrespective of the storage period prior to irradiation,

![Graphs showing PFH and LD concentrations over days](image)

**Figure 2.** Mean PFH (mg per mL) in RBC AS-1 stored for 10(A), 20(B), 30(C), and 40(D) days prior to irradiation. Plasma free hemoglobin increased in all units despite the previous period of storage prior to irradiation. No significant differences exist between the control (CTRL) and irradiated (IRRAD) units for each storage interval.

**Figure 3.** Mean LD (IU per L) in RBC AS-1 stored for 10(A), 20(B), 30(C), and 40(D) days prior to irradiation. Lactate dehydrogenase increased in all units despite the previous period of storage prior to irradiation. No significant difference exists between the control (CTRL) and irradiated (IRRAD) units for each storage interval.
no significant difference was observed between controls and irradiated pairs at all sample intervals.

Mean LD activities in control and irradiated samples are presented in figure 3. Lactate dehydrogenase activity increased in all units regardless of the previous period of storage prior to irradiation. Mean baseline values of LD at 10, 20, and 30 days of prior storage were 209 IU per L, 326 IU per L, and 569 IU per L, respectively; baseline LD in the 40 day control and irradiated units were 940 IU per L and 1075 IU per L, respectively. Irrespective of the period of storage prior to irradiation, no significant differences were observed between control and irradiated pairs at all sample intervals.

Baseline mean 2,3-DPG activity in red cells previously stored for 10 days was 0.86 +/− 0.38 μmol per mL. On the third post-irradiation day, 2,3-DPG levels were less than 0.10 μmol per mL and were undetectable at all sampling intervals to expiration. In units previously stored for 20, 30, and 40 days prior to irradiation, 2,3-DPG values were below detection limits at all sampling intervals.

Discussion

This study presents data documenting the effects of 2,000 rads of gamma irradiation on RBC previously stored for variable periods before irradiation and subsequently returned to storage. Irradiated units demonstrated increased potassium permeability with maximum values of approximately 60 to 70 mmol per L, obtained at variable intervals following irradiation. On the other hand, at expiration (42 days), all controls contained approximately 40 to 50 mmol per L of K+. The age of RBC at irradiation shows an inverse relationship to the period of time required for the cells to reach maximum potassium concentrations. For example, RBC irradiated immediately following collection exceed 60 mmol per L after 21 days of storage; units stored for 10, 20, and 30 days prior to irradiation exceeded 60 mmol per L within 10, 7, and 11 days, respectively. It is our suggestion that the maximum potassium values obtained are a function of the Na+-K+ pump, red cell porosity, and the microenvironment of the blood bags. While it is known that red cell membrane Na+-K+ shift is temperature dependent and maximal at 4°C, irradiation leads to increased permeability to Na+ and K+ by altering sulphydryl groups on membrane surfaces and within the cytoplasm. The slowed Na+-K+ shift in aged (greater than 30 days prior storage) RBC may reflect a pH dependent phenomenon.

The age of the RBC at irradiation is inversely related to the period of time for the units to exceed 50 mmol per L, the expected level for K+ in RBC AS-1 at 42 days of shelf-life. For freshly collected, irradiated and stored units, K+ exceeds 50 mmol per L at 12 days post-irradiation; those irradiated after 10 and 20 days of storage exceed 50 mmol per L within eight and four days, respectively. Moore and Ledford have shown that irradiation has little to no effect on red cell membrane Na+-K+ shift is temperature dependent; RBC CPDA-1 stored for more than seven days following irradiation have significantly increased levels of PFH compared to the paired controls. No significant difference in PFH has been noted for irradiated RBC collected in AS-1 and stored to expiration.

Because extracellular K+ load may be a significant clinical problem in selected patient populations, the additional plasma K+ concentration in aging, previously irradiated RBC must be considered. Issues regarding the efficacy of routinely washing irradiated red cells and the potassium load in small volume transfusions have been reviewed. Although
Irradiation immediately prior to transfusion is ideal, this may be impractical and unavailable in many transfusion service settings. Some transfusion services have arbitrarily established a post-irradiation storage life not to exceed five or seven days, while others have not delineated a post-irradiation shelf-life. This study documents the enhanced K+ leakage in irradiated aging RBC and suggests that prior to the establishment of a definitive post-irradiation storage life future investigation should include in vivo cell survival and red cell function studies after transfusion of variably aged, irradiated RBC.

References