Effects of Irradiation on Red Cells Stored in CPDA-1 and CPD-ADSOL (AS-1)*

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ABSTRACT

Red blood cells (pRBC) collected in citrate, phosphate, dextrose, adenine-formula 1 (CPDA-1) and citrate, phosphate, dextrose-adrenaline, manitol saline solution (CPD-ADSOL [AS-1]) anticoagulants are increasingly being stored for variable periods in transfusion service inventories following irradiation. While anecdotal reports of increased K+ following irradiation and storage have recently appeared in the literature, concomitant in vitro biochemical changes resulting from differences in anticoagulants have not been reported. Utilizing two venipunctures, two units each of 225 mL of blood from five volunteers were collected in anticoagulant-adjusted CPDA-1 and AS-1 bags. Within two hours of collection, each unit was equally divided. One of each pair was irradiated (2000 rads). Samples were analyzed on Days 0, 1, 3, 7, and every seven days to expiration. Irradiation resulted in a 2.3 fold increase in K+ during the first seven days of storage for both anticoagulants, although significantly greater K+ levels were observed in the CPDA-1 pairs compared to the AS-1 pairs. Comparison of glucose utilization, plasma free hemoglobin, 2,3-diphosphoglycerate (2,3-DPG) and lactate dehydrogenase between control and irradiated CPDA-1 and AS-1 pairs and between anticoagulants were documented.

Introduction

Irradiation of blood and cellular blood components prior to transfusion has become an accepted practice to prevent graft-versus-host disease (GVHD) in immunodeficient patients, and, more recently, in recipients of blood products from first degree relatives. With the growing demand for irradiated packed red blood cells (pRBC), an increasing number of these units have not been transfused to the intended recipient and have been returned to transfusion service inventories for transfusion to a different recipient at a later date. Prior to transfusion, these units have variable shelf lives and undergo changes in the in vitro microenvironment in both the red cell and the extracellular fluid. While, in vitro chemical changes in stored blood have been well documented,
only limited data are available on the in vitro biochemical changes in freshly collected irradiated pRBC or previously stored pRBC, then irradiated and subsequently stored before utilization. Button et al\textsuperscript{2} reported no significant difference for multiple analytes in citrate, phosphate, dextrose (CPD) anticoagulated whole blood and pRBC that were stored for 21 days and irradiated with doses of 5,000, 10,000, or 20,000 rads with sample collection shortly following irradiation. This study used non-paired controls and did not address the issues of chemical changes resulting from prolonged storage subsequent to irradiation. Moore and Ledford\textsuperscript{10} were the first to report on the in vitro changes in CPDA-1 anticoagulated RBC that were irradiated with 4,000 rads and assayed for multiple analytes at designated intervals until expiration. However, the extracellular potassium concentration (K\textsuperscript{+}) was not among the analytes assayed.

Anecdotal reports\textsuperscript{17,19} of elevated K\textsuperscript{+} in irradiated, stored pRBC, have recently appeared in the literature. Although K\textsuperscript{+} levels are significantly increased with prolonged storage in irradiated units, in vitro changes resulting from differences in commonly used anticoagulants and/or nutritive solutions have not been investigated.

This study documents a comprehensive investigation of in vitro effects of 2,000 rads of irradiation on freshly collected paired control and irradiated pRBC at designated storage intervals. It provides a comparison of these in vitro effects with regards to two conventionally used anticoagulants, CPDA-1 and CPD-ADSOL (AS-1).

Materials and Methods

Collection and Manipulation of Blood Donations

A total of 450 mL of whole blood was collected with two antecubital venipunctures from five caucasian male volunteer donors who had not given blood for at least four months. A volume of 225 mL was collected in triple AS-1 system plastic bags\textsuperscript{*} from which one-half of the CPD anticoagulant had been removed. An equal volume of 225 mL was collected in quadruple CPDA-1 system plastic bags\textsuperscript{*} following removal of one-half of the anticoagulant. Each volume of whole blood was given a unique donor number for ease of data collection. Informed consent was obtained as prescribed by the protocol approved by our Institutional Review Board. Routine serologic testing was performed and the results were provided to each donor.

All ten units were centrifuged at 4994 g\textsuperscript{+} for five minutes at 4°C. Platelet-poor plasma (PPP) was removed from each unit prior to further manipulation. An approximate volume of 100 mL of PPP was removed from the five CPDA-1 units, yielding a volume and hematocrit in each parent bag of 150 to 160 mL and 60 to 70 percent, respectively. All but approximately 10 to 15 mL of PPP were expressed from the five units collected in CPD, with 50 mL of ADSOL preservation solution added into the parent bags. This provided a volume and hematocrit in each parent bag of approximately 150 to 160 mL and 55 to 60 percent, respectively.

The contents of each of the 10 units were mixed and equally divided between the parent bag (controls) and an attached transfer bag (irradiated), and separated. Sterile sample site couplers were aseptically adapted to each bag and all sampling was performed under a laminar flow hood. All units were stored at 1 to 6°C for 42 days. Baseline sampling was obtained within two hours of blood donation. Each of the complimentary transfer bags was irradiated with 2,000 rads of

\* Fenwal Laboratories, Deerfield, IL 60015.
† RC-3B Sorvall, Dupont Co., Newton, CT 06470.
gamma irradiation.† Samples were obtained from each control and paired irradiated unit at 30 minutes (Day 0) post irradiation. Subsequent samples were obtained on post collection Days 1, 3, 7, 14, 21, 28, 35, and 42. Cultures were obtained on Day 7 and 42, and no growth was observed in all cases after seven days of incubation.

After 30 gentle inversions, aliquots of 0.5 mL of resuspended blood were obtained in tuberculin syringes, adapted with 16 gauge needles, and placed on ice prior to pH-blood gas analysis, hemoglobin, and hematocrit determination. Blood was also collected via 10 mL syringes adapted with 16 gauge needles and separated into 5.5 mL and one mL aliquots. The supernatant, collected from the 5.5 mL aliquot after centrifugation at 2,000 g for 15 minutes, was assayed for Na+, K+, glucose, creatinine, plasma free hemoglobin (PFH), and lactate dehydrogenase (LD). A volume of three mL of eight percent trichloroacetic acid was added to the one mL aliquot, incubated at room temperature for 10 minutes and centrifuged for separation of the clear supernatant and sediment. The protein free supernatant for 2,3-DPG determination was immediately frozen at −70°C and subsequently assayed in batch mode.

**ROUTINE LABORATORY METHODS**

The pH, pCO₂, and pO₂, were determined by ion-selective electrode technology.§ HCO₃⁻ and O₂ saturation were calculated values at P50.

Whole blood hemoglobin and calculated hematocrit were determined with a quantitative, automated hematology analyzer.†

Supernatant Na⁺, K⁺, glucose, and creatinine were measured using a discrete chemistry analyzer¶. Na⁺ and K⁺ were determined by ion-selective electrodes. Elevated K⁺ levels were reanalyzed by the same method using an appropriate calibration curve. Glucose levels were obtained by a glucose oxidase-oxygen rate method. Creatinine determinations were made using the Jaffe reaction.

Supernatant (free) hemoglobin levels were determined by the differential spectrophotometric analysis and calculated at delta 415 and 450 nm absorbance.

Protein-free supernatants for 2,3-DPG determinations were assayed by the method of Rose and Liebowitz;** the reference interval is 1.6 to 2.6 µmol per mL.

Total lactate dehydrogenase (LD) was determined by the modified procedure of Gay, McComb and Bowers.††

Aerobic cultures were performed by a non-radiometric, automated bacterial detection system.‡‡

The data were analyzed with paired “t” test at each assay interval. All significant levels were set at α = 0.05.

**RESULTS**

Representative raw data for multiple analytes assayed over the storage life of CPDA-1 control and paired irradiated units and AS-1 control and paired irradiated units are shown in table I. This represents analytical data at timed intervals for CPDA-1 and AS-1 units collected from one donor via two venipunctures at a single blood donation. Samples for analysis in the Day 0 column were obtained prior to subsequent irradiation of the respective CPDA-1 and AS-1

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† GammaCell 1,000, Atomic Energy of Canada Limited, Radiochemical Co., Kanata, Ontario, Canada.
§ IL 1301, Instrumentation Laboratories, Lexington, MA 02173.
¶ Coulter T-540, Coulter Corp., Hialeah, FL 33010.
¶¶ Astra-8, Beckman Instruments, Brea, CA 92621.
** Sigma Chemical Co., St. Louis, MO 63173.
†† Monarch 2000, Instrumentation Laboratory, Lexington, MA 02173.
‡‡ Pedi-Plus/Bactec HPS-660 Becton-Dickinson Diagnostic Instrument System, Towson, MD 21204.
TABLE I

Data From a Single Donor Sample With Designated Anticoagulants Under Experimental Criteria

<table>
<thead>
<tr>
<th>DAY</th>
<th>CPDA-1 CONTROL</th>
<th>CPDA-1 IRRADIATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+</td>
<td>170 169 159 152 143 137 134 131 125</td>
<td>169 158 145 132 122 118 117 114 111</td>
</tr>
<tr>
<td>K+</td>
<td>4.7 10 17 30 43 56 66 72 71</td>
<td>4.6 19 36 54 66 74 83 82</td>
</tr>
<tr>
<td>PFH</td>
<td>3.2 42 80 95 197 280 464 560 NA</td>
<td>30 37 71 112 202 299 468 601 NA</td>
</tr>
<tr>
<td>DPG</td>
<td>18 18 17 15 1.5 1.0 0.5 0.4 0</td>
<td>2.8 2.9 2.9 2.4 1.1 0.3 0.3 0</td>
</tr>
<tr>
<td>LD</td>
<td>101 128 152 184 307 475 685 936 973</td>
<td>102 120 138 176 298 419 565 847 980</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAY</th>
<th>CPD-ADSOL (AS-1) CONTROL</th>
<th>CPD-ADSOL (AS-1) IRRADIATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na+</td>
<td>153 146 140 135 133 130 124</td>
<td>153 146 137 129 120 117 115 114 111</td>
</tr>
<tr>
<td>K+</td>
<td>2.1 13 24 40 50 57 63 64 70</td>
<td>2.1 13 24 40 50 57 63 64 70</td>
</tr>
<tr>
<td>PFH</td>
<td>11 18 37 59 93 152 201 292</td>
<td>14 22 43 52 101 163 299 519</td>
</tr>
<tr>
<td>DPG</td>
<td>2.9 2.9 2.7 1.6 0.5 0.3 0.2 0.1 0</td>
<td>2.8 2.9 2.5 1.3 0.3 0.2 0.1 0</td>
</tr>
<tr>
<td>LD</td>
<td>33 31 46 58 76 129 199 198 442 757</td>
<td>37 49 68 86 142 222 339 558 723</td>
</tr>
</tbody>
</table>

Na+ (mmol/L)  K+ (mmol/L)  DPG = 2,3 diphosphoglycerate (μmol/mL)  PFH = Plasma free hemoglobin (mg/dL)  LD = Lactate dehydrogenase (IU/L)

paired units. Samples collected after 30 minutes of irradiation were not statistically different from the baseline (Day 0) results for all analytes and were not included in table I. Creatinine, total hemoglobin and hematocrit were assayed at the designated intervals and provide evidence that the intrinsic conditions within the units did not change (table II).

Serial mean K+ for control and irradiated units collected in CPDA-1 and AS-1 RBCs are shown in figure 1. The mean baseline K+ in the CPDA-1 units was 4 mmol per L, while that of the AS-1 units was 1.5 mmol per L. The concentration of K+ in the CPDA-1 and AS-1 irradiated units during the first seven days of storage was approximately 2.3 times greater than the paired controls. However, the CPDA-1 irradiated units had a significantly greater concentration of K+ than the AS-1 irradiated units. From approximately Day 7 until shelf-life expiration the loss of K+ was constant for each anticoagulant. The mean sum of Na+ and K+ for both anticoagulants is shown in figure 2A and 2B. There are no significant differences in the mean sum of measured electrolytes between the paired CPDA-1 or the paired AS-1 units. However, the sum of Na+ and K+ in the CPDA-1 units was approximately 25 mmol per L greater than the sum of measured electrolytes in the AS-1 units at each interval; a constant effect owing to differences between the two anticoagulants. The standard deviation (SD) for the combined measured electrolytes for the CPDA-1 units did not

TABLE II

Comparison of Hemoglobin, Hematocrit, and Creatinine in paired CPDA-1 and AS-1 Control and Irradiated Units From a Single Donor During SAMPPING INTERVALS

<table>
<thead>
<tr>
<th>CPDA-1</th>
<th>CPD-ADSOL (AS-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Irradiated</td>
</tr>
<tr>
<td>CRT</td>
<td>1.0 ± 0.07</td>
</tr>
<tr>
<td>HCT</td>
<td>67.7 ± 3.0</td>
</tr>
<tr>
<td>HGB</td>
<td>22.4 ± 1.3</td>
</tr>
</tbody>
</table>

CRT = Creatinine (mg/dL)  HCT = Hematocrit (Percent)  HGB = Hemoglobin (mg/dL)  (N = 10, Mean ± SD)
exceed ±6.8 mmol per L at any sampling interval, and the SD for the AS-1 unit did not exceed ±4.0 mmol per L.

Representative pH measurements from a single donor are included in table I. There was no significant difference in blood pH between the paired control and irradiated CPDA-1 or AS-1 units; however, the pH change for the anticoagulants differed significantly over the intervals studied. The mean values at any sampling interval are within 0.02 pH units of each other. Baseline pH values for both anticoagulants were slightly less than 7.0. The mean pH of the CPDA-1 units at Day 35 was 6.44, whereas the mean pH of the AS-1 units at Days 42 was 6.30. The partial pressure of oxygen (pO₂) was well maintained throughout the shelf-life for all units and provides evidence of viability.

The pRBC hemolysis data in control and irradiated samples during storage in both anticoagulants are presented in figure 3. Plasma free hemoglobin (PFH) increased over time for all samples. However, hemolysis measured as PFH was greater in the irradiated and non-irradiated CPDA-1 units compared to AS-1 units. The CPDA-1 units show no significant difference between control and irradiated units through Day 3 of storage; from Day 7 until expiration, the rate of hemolysis in the irradiated units is significantly greater than the controls. The AS-1 units show no significant difference between the control and irradiated pairs at all sample intervals.

The mean concentration of glucose in CPDA-1 and AS-1 paired units is presented in figure 4. There was no significant difference between the irradiated and control units for each anticoagulant. Glucose utilization was calculated as the difference between glucose concentrations at baseline and a given post-irradiation date. The CPDA-1 control and irradiated units utilized 390 mg per dL and 375 mg per mL of glucose, respectively, after 35 days of storage. The AS-1 control and irradiated units utilized 274 mg per dL and 268 mg per dL of glucose respectively, after 42 days of storage.

The mean activity of 2,3-DPG in control and paired irradiated cells is shown in figure 5. The baseline mean activity of 2,3-DPG in CPDA-1 and AS-1 units was 2.6 ± .3 μmol per mL; 2,3-DPG activity in all units began a significant decline by Day 7 and reached levels less than 0.5 μmol per mL by Day 28. The CPDA-1 paired units showed a significant differ-
FIGURE 2A. These data demonstrate the relative increase and decrease of K⁺ (mmol per L) and Na⁺ (mmol per L) for control and irradiated samples with CPDA-1 anticoagulant. The sum of K⁺ and Na⁺ is shown.

FIGURE 2B. These data demonstrate the relative increase and decrease of K⁺ (mmol per L) and Na⁺ (mmol per L) for control and irradiated samples with AS-1 anticoagulant. The sum of Na⁺ and K⁺ is shown.

ence (P < 0.025) only on Day 28; there were no significant differences between control and irradiated units at other sampling intervals. The AS-1 paired units, showed no significant differences in control and irradiated units at any sampling interval.

The mean LD (IU per L) activity in control and paired irradiated cells for both anticoagulants is presented in figure 6. Baseline LD activity was considerably less than 200 IU per L for all units; at Day 14 LD activity was substantially greater than 200 IU per L for all units. Peak values of approximately 1000 IU per L were reached for all units by Day 42. There was no significant difference between the CPDA-1 pairs or the AS-1 pairs; however, on the average, the CPDA-1 units contained approximately
50 IU per L greater activity of LD at each sample interval than did the AS-1 units.

**Discussion**

This study provides a comprehensive investigation of in vitro effects of irradiation on freshly collected, irradiated packed red blood cells over the storage life of the cells; a comparison is also made of in vitro effects resulting from differences in anticoagulants. Donor venous blood was collected in two different anticoagulants at a single donation, thereby avoiding donor variables resulting from sequential (56 days) whole unit collections. Blood units were irradiated with 2,000 rads, a dose sufficient to prevent transfusion-related GVHD at our hospital.

The critical target for radiation within a cell is recognized to be deoxyribonucleic acid (DNA); however, significant perturbations of cell membranes also occur. Red blood cells, although devoid of a nucleus, are the site of continuous
metabolic activity. At physiologic temperatures, red cells maintain a concentration gradient of cations against their environment. In the intact red cell, K⁺ loss and Na⁺ gain is temperature dependent and maximal at 4°C.⁴,¹³,²³,²⁸ Irradiation leads to increased permeability to Na⁺ and K⁺ by alternating sulfhydryl groups on membrane surfaces and within red cells.³,²¹,²² Hemolysis occurs at high irradiation doses.¹³ Altered electrophoretic mobility of membrane proteins and glycoproteins, reflecting changes in surface charge, occurs following irradiation.¹⁸ Structural modifications involving peroxidation of unsaturated lipids of the membrane also occur following irradiation.¹⁶,²⁷ Shapiro and Kollman²¹ observed Na⁺ uptake and K⁺ loss at 2,000 rads at 20 hours following irradiation nearly double that of the controls. These findings were substantiated by us during the first seven days after irradiation irrespective of the anticoagulant. For each anticoagulant,
the $K^+$ loss after the seventh day of storage remained constant compared to its respective controls. This would suggest that red cell membrane irradiation damage progresses for a limited number of days, after which membrane repair may occur. The difference in $K^+$ loss between anticoagulants is significant (figure 1). This difference is clearly not dilutional; rather it appears to result from the composition of the anticoagulant and/or nutritive-preservative solution. The presence of adenine does not appear to influence potassium shifts during storage;\textsuperscript{1,24} adenine compounds affect the transport of hydrophilic molecules across red cell membranes, and the effect is concentration dependent.\textsuperscript{7}

Since glucose utilization between irradiated and non-irradiated units for each anticoagulant was not significantly different, the cation exchange across the membrane would appear to be non-glucose dependent. It has been shown that electrolyte disturbances after irradiation involve the entrance of $Na^+$ in amounts nearly equivalent to the $K^+$ leaving the cells.\textsuperscript{5,23} Our data support these findings in red blood cells stored up to 35 and 42 days, respectively. However, the data suggest that another cation, most probably $H^+$, enters the cells; therefore, the $Na^+-K^+$ shift is temperature dependent, and may be pH dependent.

The decrease in 2,3-DPG activity in this study is similar to those seen from previous reports.\textsuperscript{10,12,15} Irradiation did not result in a significant difference in 2,3-DPG levels relative to the controls for either the CPDA-1 units, except on Day 28, or the AS-1 units. Moore, et al\textsuperscript{11} reported slightly lower 2,3-DPG in adenine-containing units (CPD vs. CPDA-1). However, when the data were reanalyzed by repeated measures analysis of variance, 2,3-DPG levels in the CPDA-1 units were not significantly different from the CPD units.\textsuperscript{12} Our data suggest, however, a slightly greater decrease in 2,3-DPG in the AS-1 units compared to the CPDA-1 units. Irradiation resulted in an increase in PFH in the CPDA-1 units from Day 7 through the end of storage, with concentrations during this period approximately 20 to 25 percent greater than the controls at the intervals studied. Since no significant difference in PFH in the AS-1 units was observed, the composition of the anticoagulant-nutritive solution may provide a limited protective role against irradiation effect with respect to PFH increase.

Lactate dehydrogenase (LD) activity, to our knowledge, has not been previously studied in irradiated pRBCs. There is a constant increase during storage and large amounts are noted at the end of storage; in general, PFH levels parallel LD activity following irradiation and storage. An unexpected finding in this study was the final pH in both anticoagulants. Although no differences were found for blood–pH between the control and paired irradiated units for each anticoagulant, the final mean pH in the CPDA-1 units at Day 35 was 6.44, and 6.3 at Day 42 in the AS-1 units. Published data report a pH of 6.6 for CPDA-1 and AS-1 units at Day 35 and 49, respectively.\textsuperscript{6} Our findings may be related to acid-base shifts reflecting the storage of small volumes of pRBC in blood bags with gaseous permeation (unpublished data).

The clinical interpretation and applications resulting from this study are not resolved. For blood centers offering red cell irradiation, prolonged storage following irradiation may expose selected patient populations to a significant $K^+$ load following transfusion. Since our data show clear differences in $K^+$ levels over time between two anticoagulants, the selection of anticoagulant and post irradiation storage period may become significant considerations in the transfusion of irradiated red cell products, and may require differing post irradiation storage dates for neonates and adults. In our opinion, our study indicates the need for continued investigation into the effects of
irradiation on pRBC, the role of anticoagulants in the metabolic changes observed post irradiation, and the clinical significance of metabolic derangements in irradiated blood collected in a variety of anticoagulant solutions.

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References


