Effect of Gamma Irradiation on the \textit{in vivo} Recovery of Stored Red Blood Cells*†

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ABSTRACT

Gamma irradiation of blood components is used to prevent posttransfusion graft versus host disease. This process has been demonstrated to cause an increase in the permeability of the red blood cell membrane to potassium and sodium. Because of this red cell membrane lesion, it is important to investigate the effect of irradiation on the posttransfusion recovery of stored red blood cells.

In the present study, the 24-hour posttransfusion recovery of AS-1 red cells irradiated with 30 Gy one or 14 days after collection and stored for a total of 35 days was compared to the recovery of unirradiated red blood cells stored for 35 days.

There was no significant difference in the mean 24-hour posttransfusion recovery of \textsuperscript{51}Cr labeled red blood cells among any of the groups studied. Each group had a mean recovery >75 percent. The mean potassium and hemoglobin concentrations at the end of 35 days of storage were significantly higher in both of the irradiated groups compared to the unirradiated group, but were not significantly different from each other.

Under the conditions of this study, gamma irradiation did not significantly affect the 24-hour posttransfusion recovery of red blood cells stored for 35 days.

Introduction

Gamma irradiation of blood components to prevent posttransfusion graft versus host disease in patients who are immunocompromised or who are receiving blood from relatives has become an increasingly common practice.\textsuperscript{4} A number of reports have documented that this irradiation results in an increase in the concentration of extracellular potassium.\textsuperscript{1,2,6,9,10,12,13,20,21} This has been shown to be due to an increased permeability to sodium and potassium of the red blood cell membrane lipid bilayer.\textsuperscript{5}

Because of this red cell membrane lesion, it is important to investigate the effect of gamma irradiation on the \textit{in vivo} recovery of stored red blood cells. The
United States Food and Drug Administration (FDA) has determined recently that between 15 and 20 percent of red cell inventory was more than 28 days old, and the Council of Community Blood Center members ascertained that 20 percent of red cell components were more than 28 days into their shelf life at the time of transfusion. Since many hospitals do not have facilities that permit on site irradiation of blood components and, even for hospitals that do, it is often not possible to predict when a particular unit will need to be transfused. Units of blood may need to be stored for an indefinite period after irradiation.

The purpose of the present study was to determine the 24 hour posttransfusion recovery of red blood cells irradiated one or 14 days after collection and stored for a total of 35 days. This storage interval was selected to reflect a real-life situation. The specific aims of the study were to ascertain whether or not the 24 hour recovery of such units met or exceeded the 75 percent recovery established as acceptable for red cell transfusion by the FDA and whether or not the recovery was comparable to red blood cells that had not received irradiation.

Materials and Methods

Four hundred fifty ml of whole blood were obtained in PL-146 containers from 12 individuals, each of whom provided informed consent and who met all of the criteria for blood donation of the Standards of the American Association of Blood Banks. The investigation was approved by the Human Investigation Committee of the University of Virginia Health Sciences Center School of Medicine and in accord with an assurance filed with, and approved by, the Department of Health and Human Services.

The AS-1 preserved red blood cells were prepared from each of the units collected. These units were then placed into storage at 4°C. Four units (Group A) were selected to receive 30 Gy of gamma irradiation after 24 hours of storage and were then returned to 4°C storage for an additional 34 days. Four other units (Group B) were selected to receive 30 Gy of gamma irradiation after 14 days of storage. These units were then returned to 4°C storage for an additional 21 days. The remaining four units (Group C) were left undisturbed for 35 days.

After 35 days of storage, autologous 24 hour red blood cell recovery studies with chromium-51 (51Cr) radiolabelled red blood cells were performed using the procedure of Moroff et al. Red blood cell mass determinations were made simultaneously using technetium-99m (99mTc) radiolabelled autologous red blood cells by Heaton et al.’s modification of the method of Beutler and West and also extrapolated from 51Cr recovery.

Supernatant potassium concentrations were determined by ion-specific electrode. Plasma hemoglobin concentrations were determined spectrophotometrically.

Red blood cell recovery, extracellular potassium and plasma hemoglobin concentrations were compared using unpaired t tests.

Results

In table I are listed the 24 hour red blood cell recovery in each of the 12 donors calculated from the red cell mass determined by 51Cr or 99mTc. There was no significant difference in the mean 24 hour red blood cell recovery among any of the groups. In each of the groups, mean
In table III are listed the plasma hemoglobin concentration at 35 days of storage for each of the donors. The mean plasma hemoglobin concentrations in Group A and Group B were each higher than the mean in Group C (A vs. C p < 0.005, B vs. C p < .02) but were not different from each other.

**Discussion**

Gamma irradiation of cellular blood components is the only procedure that has been demonstrated to prevent post-transfusion graft versus host disease.\(^3,4\) A recently published survey conducted in 1989 determined that more than 10 percent of cellular blood components were irradiated prior to transfusion despite the fact that only approximately 12 percent of hospital blood banks and transfusion services had on site facilities for this service.\(^4\) Since this survey was conducted, an additional American Association of Blood Bank (AABB) Standard has suggested that all cellular blood components from first-degree relatives should be irradiated prior to transfusion,\(^24\) and a recent analysis suggested that such components from all relatives may need to be irradiated.\(^15\) Thus, the proportion of blood components undergoing gamma irradiation presumably has continued to

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**TABLE I**

24 Hour Red Blood Cell Recovery (%)
Calculated from Red Blood Cell Mass
Determined by \(^{51}\)Cr or \(^{99m}\)Tc

<table>
<thead>
<tr>
<th></th>
<th>(^{51})Cr</th>
<th>(^{99m})Tc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1. 73.8</td>
<td>74.3</td>
</tr>
<tr>
<td></td>
<td>2. 87.3</td>
<td>89.2</td>
</tr>
<tr>
<td></td>
<td>3. 69.5</td>
<td>72.3</td>
</tr>
<tr>
<td></td>
<td>4. 81.3</td>
<td>82.9</td>
</tr>
<tr>
<td>Mean (S.D.)</td>
<td>78.0 (6.8)</td>
<td>79.7 (6.8)</td>
</tr>
</tbody>
</table>

**TABLE II**

Extracellular Potassium (MEq/L)
at 35 Days of Storage

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>64</td>
<td>62</td>
<td>54</td>
</tr>
<tr>
<td>2.</td>
<td>65</td>
<td>77</td>
<td>47</td>
</tr>
<tr>
<td>3.</td>
<td>73</td>
<td>69</td>
<td>55</td>
</tr>
<tr>
<td>4.</td>
<td>71</td>
<td>76</td>
<td>44</td>
</tr>
<tr>
<td>Mean (S.D.)</td>
<td>68 (3.8)</td>
<td>71 (6.0)</td>
<td>50 (4.6)</td>
</tr>
</tbody>
</table>

**TABLE III**

Plasma Hemoglobin Concentrations (mg/dl) and Percent Lysis at 35 Days of Storage

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb Lys</td>
<td>266 0.7</td>
<td>323 0.8</td>
<td>158 0.4</td>
</tr>
<tr>
<td>Lysis</td>
<td>302 0.8</td>
<td>288 0.8</td>
<td>189 0.5</td>
</tr>
<tr>
<td>Hgb Lys</td>
<td>388 0.9</td>
<td>300 0.7</td>
<td>201 0.5</td>
</tr>
<tr>
<td>Lysis</td>
<td>300 0.7</td>
<td>455 1.1</td>
<td>150 0.4</td>
</tr>
<tr>
<td>Mean (S.D.)</td>
<td>314 (0.8)</td>
<td>342 (0.9)</td>
<td>175 (0.5)</td>
</tr>
</tbody>
</table>

Group A = 30 Gy at 24 hours, then stored for 34 days. Group B = 30 Gy at 14 days, then stored for 21 days. Group C = stored for 35 days.
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increase. Although the transfusion of blood cells exposed to gamma radiation is believed to be safe, a number of reports have determined that irradiation of components containing red cells results in an increase of the concentration of extracellular potassium.\textsuperscript{1,2,6,9,10,12,13,20,21} The results of this study confirm these observations (table II).

Because many facilities do not have the on site capability for irradiating blood components and because changes in clinical circumstances or unforeseen events such as the postponement of surgery often make it hard to predict when a particular unit will need to be transfused, irradiated blood components may need to be stored for some period of time prior to transfusion. Because of the increased accumulation of plasma potassium and hemoglobin in such units, it is important to document the effect of gamma irradiation on the \textit{in vivo} recovery of stored red blood cells.

As noted previously, a large proportion of blood collected in the United States is transfused after four weeks of storage.\textsuperscript{7} Consequently, the current authors elected to study red blood cells that had been subjected to extended storage for 35 days. Two different times for irradiation were selected. The first to reflect the possibility that blood would be irradiated in a collection center shortly after collection (either to develop an inventory of irradiated blood or on a specific unit, such as one donated by a relative, for a hospital without irradiation capability) and then stored either in the center or the hospital prior to transfusion. The second to reflect a situation in which the blood would be irradiated either in the blood center or the hospital after a period of time had elapsed since the collection but then would have to be stored for an additional period of time. A dose of 30 Gy was selected to insures that all areas of the blood component received at least 25 Gy. This dose was recommended recently as the most appropriate\textsuperscript{17} and is the minimum dose required by the most recent edition of the AABB Standards,\textsuperscript{23} since lower doses could conceivably be less effective in the prevention of graft versus host disease.\textsuperscript{18,19}

The results of this study suggest, with the conditions used, 24 hour red cell recovery is not compromised significantly by this dose of gamma irradiation. Furthermore, mean 24 hour red cell recovery exceeded the 75 percent level deemed acceptable by the FDA. Further studies will be required to extend and confirm these findings.

In the present study, the mean red blood cell mass calculated from the \textsuperscript{51}Cr recovery was 1,338 ml. The mean red cell mass determined from the \textsuperscript{99m}Tc was 1,384 ml. The slightly increased red cell mass with \textsuperscript{99m}Tc has been reported previously.\textsuperscript{5,14,22} Friedman and colleagues have reported that irradiation of red blood cells stored in AS-3 and irradiated with 20 Gy one day after collection had somewhat reduced 24 hour \textsuperscript{51}Cr recovery at 21 and 28 days compared to unirradiated blood that still, however, exceeded 80 percent.\textsuperscript{10} Davey and colleagues reported significantly reduced posttransfusion 24 hour red cell recovery in AS-1 units that had been stored for 42 days after having been exposed to 30 Gy shortly after collection compared to unirradiated blood.\textsuperscript{9} The recovery in the irradiated units did not achieve the 75 percent level required by the FDA. The combined data of Friedman et al., Davey et al., and this study show an overall concordance insofar as survival of stored irradiated units was 80.7 percent at 28 days of storage,\textsuperscript{10} 78 percent at 35 days of storage, and 68.5 percent at 42 days of storage.\textsuperscript{9}

Significantly increased plasma hemoglobin concentrations were detected by us in the irradiated units in accord with the data of Davey et al. Jeter and colleagues found a significant increase in plasma hemoglobin in irradiated
CPDA-1 units but not in irradiated AS-1 units.\textsuperscript{12} Despite the increased hemoglobin, our results suggest a mean of less than one percent hemolysis in the irradiated units. Nevertheless, the increased hemoglobin may indicate red blood cell membrane damage in addition to increased cationic permeability.

The transfusion of gamma irradiated red blood cell components is a common practice. The data presented in this report suggest that the transfusion of red blood cells subjected to 30 Gy of gamma irradiation on day one or day 14 of storage have acceptable 24 hour posttransfusion recovery after a total of 35 days of storage that, in this small series, was comparable to the recovery of unirradiated red cells.

\textbf{Acknowledgments}

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\textbf{References}


