The Effect of Metal Chelators on Lipid Peroxidation in Irradiated Erythrocytes

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ABSTRACT

Prophylactic irradiation of blood and blood components is accepted practice in order to prevent graft-versus-host disease from infused lymphocytes. Irradiation, however, results in increased red cell potassium (K+) loss, along with other possible effects that may affect red cell function and viability. Lipid peroxidation (LP), a process initiated by the production of oxygen free radicals, is increased in red cells in the presence of reactive iron species and various heme moieties. In this report, it is noted that not only is plasma K+ significantly increased following blood irradiation, but LP is also increased compared with paired non-irradiated blood samples. Furthermore, various metal chelators significantly reduce LP in the irradiated samples. These chelators also significantly reduced the rate of cellular K+ loss during the four day 37°C incubation period.

This study further suggests that the addition of selected metal chelators may be effective in both irradiated and non-irradiated stored blood by improving the function and viability of transfused erythrocytes.

Introduction

Prophylactic irradiation of blood and blood components prior to transfusion is recommended to reduce the risk of graft-versus-host disease in various high-risk patients. These include those receiving (1) bone marrow grafts, (2) in utero fetal transfusions, and (3) intensive chemotherapy, as well as in (4) immunodeficient patients, and (5) directed donations from first-degree relatives. However, in a recent survey, 87.7 percent of blood banks did not have on-site facilities for gamma irradiation. Blood banks lacking these facilities must make arrangements with outside blood centers or hospitals for irradiation services. Logistic complications involving the use of outside facilities for irradiation services often require blood banks to store an inventory of irradiated blood in order to meet fluctuating demands with adequacy. As the clinical indications for irradiated blood expand, the need to store more of these products is also likely to expand. As a result, the best methods for storing irradiated blood need to be delineated.

In this regard, several recent studies have reported significantly increased plasma potassium (K+) concentrations in...
stored blood following red cell irradiation when compared with non-irradiated paired controls.\textsuperscript{5,12,13} Other reported post-irradiation changes include decreased levels in serum sodium, red cell adenosine triphosphate (ATP) and 2,3 diphosphoglycerate (2,3 DPG), and increased hemolysis; blood pH, methemoglobin, and glucose levels were unchanged.\textsuperscript{11}

Decreased lipid peroxidation was reported by us recently, as determined by plasma malondialdehyde (MDA) levels, in blood stored in the presence of various metal chelators and antioxidants when compared with paired controls.\textsuperscript{7,8} In particular, diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), and deferoxamine mesylate (DM) were all very effective in reducing lipid peroxidation. This positive effect of the metal chelators is presumably due to iron binding following its release from red cell breakdown, thereby preventing the production of hydroxyl free radicals (OH\textsuperscript{•}) via the Fenton and/or Haber-Weiss reactions.

This current study is an extension of these early reports in which the effect was noted of selected metal chelators added to irradiated blood by measuring plasma MDA and K\textsuperscript{+} levels and compared to paired non-irradiated and irradiated controls without added chelators.

Materials and Methods

Specimens/Procedures

Duplicate 7.0 mL blood samples, obtained from blood donors during the normal donor process, from each study group (11 duplicate samples in each group), were anticoagulated with citrate, phosphate, dextrose, adenine-formula 1 (CPDA-1) solution in the same ratio as present in normal whole blood donor containers (0.8 mL CPDA-1 added to 7.0 mL of whole blood). The samples were stored at 3°C until analysis for malondialdehyde (MDA) baseline values, always within 24 hours. Paired duplicate blood samples were irradiated just prior to analysis for MDA with 2500 rad.* Duplicate plasma MDA levels were determined by high performance liquid chromatography (HPLC) as previously reported\textsuperscript{10} with modifications with respect to the equipment used.\textsuperscript{6} Potassium (K\textsuperscript{+}) was quantified using a discrete chemistry analyzer according to the manufacturer's procedure.\textsuperscript{†} The initial K\textsuperscript{+} measurements (day 0) followed the serum/plasma procedure while subsequent measurements (days 7, 14, 18) used the urine procedure.

The blood samples were then stored at 3°C, mixed several times by gentle inversion every other day including the day of MDA and K\textsuperscript{+} analysis, and the plasma analyzed following gentle centrifugation at approximately 75 g for five minutes after 7 and 14 days. The samples were then placed in a 37°C incubator to simulate \textit{in vivo} conditions, again gently mixed by inversion several times on day 17 and immediately before MDA and K\textsuperscript{+} analysis on day 18, centrifuged at about 2500 g for five minutes, and the plasma analyzed for MDA and K\textsuperscript{+}. Thus, the control plasma samples were analyzed for MDA and K\textsuperscript{+} on days 0, 7, 14, and 18. Duplicate blood samples were irradiated on day 0; the various metal chelators were then added to their respective sample groups and analyzed in exactly the same manner. Plasma MDA and K\textsuperscript{+} concentrations were quantified on days 7, 14, and 18.

Reagents/Solutions

The phosphoric acid, thiobarbituric acid (TBA), 1,1,3,3-tetraethoxy-propane (TEP), methanol-NaOH solution, phos-
Levels of Plasma Malondialdehyde (μmol/L) Following Irradiation and Subsequent Incubation with Metal Chelators (Mean ± Standard Deviation)

<table>
<thead>
<tr>
<th>Day</th>
<th>Controls</th>
<th>Irradiated</th>
<th>DTPA + Irr</th>
<th>DM + Irr</th>
<th>EDTA + Irr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.96 (0.20)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>1.51 (0.33)</td>
<td>1.79 (0.50)a</td>
<td>1.07 (0.21)bc</td>
<td>0.75 (0.15)bc</td>
<td>1.09 (0.24)bc</td>
</tr>
<tr>
<td>14</td>
<td>1.97 (0.50)</td>
<td>2.33 (0.50)a</td>
<td>1.28 (0.37)bc</td>
<td>0.78 (0.13)bc</td>
<td>1.85 (0.35)d</td>
</tr>
<tr>
<td>18</td>
<td>5.85 (1.49)</td>
<td>7.58 (1.79)e</td>
<td>1.78 (0.57)bc</td>
<td>3.75 (0.49)bc</td>
<td>2.56 (0.36)bc</td>
</tr>
</tbody>
</table>

DTPA = diethylenetriaminepentaacetic acid  
EDTA = ethylenediaminetetraacetic acid  
Irr = deferoxamine mesylate

Test samples vs. controls:  aP < 0.05;  bP < 0.01;  cP < 0.001  
Metal chelators vs. irradiated specimens:  dP < 0.01;  eP < 0.001

Our results comparing plasma MDA in the control specimens with the irradiated group and the irradiated groups containing DTPA, DM, and EDTA are shown in table I and figure 1. With each group there was, as expected, a progressive increase in plasma MDA with time, and a significantly larger rise during the four day 37°C incubation period. Also as expected, the irradiated group alone resulted in significantly higher MDA levels than the control group owing to the radiolysis of water with subsequent increased free radical production. As in prior studies, the metal chelators were very effective in reducing MDA production; again, both DTPA and EDTA were somewhat more effective than DM. In addition, the presence of the metal chelators resulted in a more significant reduction in MDA in the irradiated samples compared with the non-irradiated controls. This finding could be of clinical significance in considering red cell viability and life-span in the transfusion of irradiated blood.

Statistical Analysis

Owing to the consistency of the MDA and K⁺ levels (MDA but not K⁺ was analyzed in duplicate), seven to eight samples were randomly chosen from each control group (total of 30 non-irradiated control specimens) and compared on days 7, 14, and 18 with the blood specimens from each of the various specific groups: irradiated without chelators; irradiated + DTPA; irradiated + DM; and irradiated + EDTA. Data computations included means, standard deviations, and the Mann-Whitney test.

Results

Our results comparing plasma MDA in the control specimens with the irradiated group and the irradiated groups containing DTPA, DM, and EDTA are shown in table I and figure 1. With each group there was, as expected, a progressive increase in plasma MDA with time, and a significantly larger rise during the four day 37°C incubation period. Also as expected, the irradiated group alone resulted in significantly higher MDA levels than the control group owing to the radiolysis of water with subsequent increased free radical production. As in prior studies, the metal chelators were very effective in reducing MDA production; again, both DTPA and EDTA were somewhat more effective than DM. In addition, the presence of the metal chelators resulted in a more significant reduction in MDA in the irradiated samples compared with the non-irradiated controls. This finding could be of clinical significance in considering red cell viability and life-span in the transfusion of irradiated blood.
As noted in other reports, plasma K⁺ levels rise rapidly in blood stored at 3° to 4°C. This is also well illustrated in our study (table II, figure 2). Furthermore, the K⁺ rise is even more striking in irradiated blood compared with non-irradiated controls, especially at 3° to 4°C. This was also the case in the irradiated specimens containing the metal chelators. Interestingly, however, there was little or no change in plasma K⁺ following the 37°C incubation time in specimens containing the chelators, especially DTPA and EDTA. In fact, the plasma K⁺ levels were decreased at day 18 compared to day 14 in the DTPA group. In any event, it is significant that in all irradiated groups there was a relative

**TABLE II**
Levels of Plasma Potassium (mmol/L Following Irradiation and Subsequent Incubation with Metal Chelators (Mean ± Standard Deviation))

<table>
<thead>
<tr>
<th>Day</th>
<th>Controls</th>
<th>Irradiated</th>
<th>DTPA + Irr</th>
<th>DM + Irr</th>
<th>EDTA + Irr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.3 (0.9)</td>
<td>-----------</td>
<td>-----------</td>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>7</td>
<td>24.2 (3.5)</td>
<td>39.9 (4.7)</td>
<td>38.5 (3.3)</td>
<td>33.9 (2.4)</td>
<td>40.6 (3.8)</td>
</tr>
<tr>
<td>14</td>
<td>32.7 (4.3)</td>
<td>45.9 (2.8)</td>
<td>45.3 (2.8)</td>
<td>41.4 (2.3)</td>
<td>46.2 (4.0)</td>
</tr>
<tr>
<td>18</td>
<td>52.1 (4.8)</td>
<td>50.5 (3.3)</td>
<td>41.1 (1.7)</td>
<td>46.2 (3.1)</td>
<td>47.2 (3.7)</td>
</tr>
</tbody>
</table>

DTPA = diethylenetriaminepentaacetic acid
EDTA = ethylenediaminetetraacetic acid
DM = deferoxamine mesylate
Irr = irradiated

Statistical significance:
Test samples vs. controls: b Not significant; c P < 0.005; a P < 0.001
Figure 2. Plasma potassium (K⁺) levels plotted against time in days. Note particularly the sharp increase in the control K⁺ levels from day 14 to 18 (37°C) while the irradiated specimens, particularly those containing diethylenetriaminepentaacetic acid (DTPA) and ethylenediamine tetraacetic acid (EDTA), are unchanged or decreased.

decrease in K⁺ following the four day incubation period compared with the non-irradiated controls (table II, figure 2).

Discussion

The infusion of blood products into patients who are immunodepressed or have immunodeficiency disease, or are undergoing bone marrow transplantation or intensive chemotherapy for specific malignancies are at risk of developing graft-versus-host disease from the infused lymphocytes. This risk can, however, be minimized by irradiating the blood products immediately before infusion as initially noted by Button et al.⁴ More recently, others¹¹ reported that by irradiating stored red cells on the day of blood collection with 4000 rads, the irradiation did not cause significant red cell biochemical or metabolic changes during storage that would lead them to suspect a difference between irradiated and nonirradiated red cells in function or viability. These conclusions were based on their measurement of red cell ATP, 2,3 DPG, methemoglobin, blood glucose, and plasma hemoglobin.

On the other hand, several studies⁵,¹²,¹³ have reported significantly increased plasma K⁺ concentrations in stored blood following red cell irradiation when compared with non-irradiated paired controls. The mechanism is reportedly by alternating sulfhydryl groups on membrane surfaces and within red cells.⁵ In addition, irradiated blood anticoagulated with CPDA-1 has a significantly greater K⁺ concentration at comparable periods of post-irradiated storage than irradiated blood anticoagulated with CPD-ADSOL (AS-1),⁵ suggesting that K⁺ levels and other parameters may also be of importance in assessing the function and viability of stored erythrocytes.

In this latter regard, the extent of lipid peroxidation (LP) in stored erythrocytes was reported by us.⁷,⁸ Furthermore, it
was noted that the production of hydroxyl free radicals (OH·) and LP could be very significantly reduced in the presence of selected iron-binding metal chelators, and to a significant but lesser degree following the addition of the natural antioxidant glutathione. In the current report, these studies were extended to stored erythrocytes following irradiation. Here, it is noted that LP is significantly increased in stored irradiated blood when compared with non-irradiated paired samples. Furthermore, these metal chelators were very effective in reducing LP when added to irradiated samples in comparison to the paired irradiated blood samples without chelators.

As noted previously, K+ is rapidly released from red cells during storage at 3° or 4°C; in our control group, an even sharper increase was noted following incubation at 37°C (table II, figure 2). Importantly, however, this rise during the 37°C incubation period was greatly slowed in the presence of the chelators, and, in the case of DTPA, the plasma K+ was actually decreased (day 14 vs. day 18). Thus, it appears that the binding of iron released from aging and/or metabolically injured red cells decreases the formation of OH· and subsequent LP, as well as other known destructive effects on cellular proteins, including enzymes. Thus, alternating sulfhydryl groups on red cell membranes and within the red cells may be less affected. In addition, the effects of OH· and LP on cellular ionic channels may be reduced by decreasing damage to the red cell lipid bilayer, thereby allowing the normal ion-exchange mechanism to function more efficiently at 37°C.

In summary, the current findings, along with those previously reported, suggest the possibility that the addition of an appropriate metal chelator to donor blood, both irradiated and non-irradiated, might be effective in further extending the function and viability of stored red cells.

References