The Effect of Desferrioxamine on Stored Erythrocytes: Lipid Peroxidation, Deformability, and Morphology*

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ABSTRACT

Acid-citrate-dextrose (ACD) was introduced in 1947 as a nutrient-anticoagulant solution to preserve stored whole blood. A decade later, the introduction of citrate-phosphate-dextrose (CPD) resulted in the successful preservation of whole blood for 21 days. In 1978, added adenine and increased glucose concentration (CPDA-1) increased storage to 35 days. More recently, other solutions such as AS-1, further increased the acceptable storage time of packed cells, but cost and inconvenience have limited their use. Several recent studies have investigated the possibility of increasing the shelf-life of whole blood by the addition of various antioxidants. In the current study a comparison was made of the effects of the metal chelator desferrioxamine on several parameters of stored red cells. Thus, measurement was made of plasma malondialdehyde levels, a measure of lipid peroxidation, red cell filtration times, a measure of red cell deformability, and red cell morphology by electron microscopy. Our results showed that adding desferrioxamine significantly decreased levels of plasma malondialdehyde (MDA) and slowed the loss of erythrocyte deformability. Although red cell morphology suggested that the treated cells maintained their normal appearance longer than untreated cells, the differences were not statistically significant. These results suggest that the addition of desferrioxamine to CPDA-1 anticoagulated blood might be effective in prolonging the viability of stored erythrocytes.

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Introduction

The introduction of acid-citrate-dextrose (ACD) in 1947 as a nutrient-anticoagulant was a major step forward in the preservation of whole blood for transfusion. In 1957, Gibson et al. reported that blood collected and stored in citrate-phosphate-dextrose (CPD) was better preserved than that collected and stored in ACD, a finding later confirmed by others. However, erythrocyte storage in these solutions results in a significant progressive loss of intracellular adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG). As a result, erythrocyte membranes become rigid and lose their deformability, oxygen exchange decreases, and the red cells lose their viability. However, the addition of adenine to CPD increases red cell 2,3-DPG and ATP, thereby partially restoring these critical properties and increasing the lifespan of the circulating erythrocytes.

Further supplementation of this formula with additional glucose (CPDA-1) has been the primary nutrient anticoagulant since 1978. Thus, the addition of adenine and increasing the concentration of glucose resulted in an increased storage time from 21 to 35 days. In an attempt to improve further red cell storage viability, several other nutrient-anticoagulant solutions have been recently introduced including CPDA-2, CPDA-3, AS-1, AS-3, and AS-5. At least two of these systems (AS-1 and AS-3) have been approved for red cell storage beyond 35 days. However, they require a second preservative solution that must be added shortly after the blood has been drawn. The solutions contain saline, dextrose, adenine, and other additives designed to enhance erythrocyte function and survival.

In 1967, Anbar and Neta reported that oxygen-derived free radicals and lipid peroxidation (LP) represented the primary mechanism of cellular injury following radiation. Thus, LP, "the oxidative deterioration of polyunsaturated fatty acids," is now a widely accepted mechanism for cellular injury and death, and has been implicated in a wide variety of diseases, as well as aging. With respect to LP and red cells, Lee reported that the addition of reduced glutathione (GSH) and ethylenediaminetetraacetic acid (EDTA) significantly decreased LP in plasma stored at 4° to 5°C. When both were added, the antioxidant effects were additive. In addition, Lachant et al. noted increasing Heinz body formation and decreased glutathione levels in stored red cells. More recently, the present authors reported significantly reduced LP in stored whole blood following the addition of various metal-binding chelators and free radical scavengers to both non-irradiated and irradiated blood. Furthermore, donors supplemented with the antioxidant vitamins C and E resulted in decreased LP in both irradiated and non-irradiated erythrocytes.

In the current study, desferrioxamine, a metal chelator, was added to CPDA-1 anticoagulated whole blood. Over time, plasma malondialdehyde, as a measure of LP, was measured and these results compared with both red cell deformability, as determined by erythrocyte filtration rates, and morphologic erythrocyte changes by transmission electron microscopy.

Materials and Methods

Specimens and Procedures

Duplicate 7.0 mL blood samples were collected from 10 healthy blood donors during the normal blood donation process. Each sample was anticoagulated with citrate-phosphate-dextrose-adenine (CPDA-1) in the same ratios as present in whole blood collection containers (7.0 mL whole blood added to 0.8 mL CPDA-1). The collection tubes were
mixed by gentle inversion immediately after filling to ensure adequate anticoagulation. The samples were stored at 3°C until they were analyzed. Baseline values were determined within 18 hours of collection for malondialdehyde (MDA), filtration time, hematologic analyses, and fixation for electron microscopy (EM). Prior to centrifugation for MDA measurements, the samples were mixed by inversion and aliquots of 0.5 mL and 200 μL were removed for EM evaluation and filtration times respectively.

**Malondialdehyde (MDA) Levels.** Duplicate plasma MDA levels were determined by high performance liquid chromatography (HPLC) as previously reported by Wong et al. The samples were then stored at 3° and 4°C and mixed every other day by gentle inversion. The samples were then centrifuged at approximately 75 g for five minutes after 7 and 14 days. The samples were then placed in a 37°C incubator for four days (day 18) to simulate the \textit{in vivo} temperature and to place added stress on the red cells. Prior to analysis, and after the previously mentioned aliquots were removed, the samples were centrifuged for five minutes at about 2,500 g.

**Filtration Studies.** The procedure described by Levander et al. was followed for the filtration analyses. Here, a suction flask fitted with a 15 mL funnel filter (25 mm effective surface filtration area) was attached to a water manometer and two siphon bottles by thick-walled vacuum tubing. The polycarbonate filters had a 3 μm pore diameter.* The 200 μL aliquot of whole blood was added to 10 mL filtration buffer just prior to filtration. A 10 cm water vacuum was applied to the system after which 2.0 mL of the blood/buffer solution was placed into the funnel and timed by means of an electronic stop watch. This procedure was performed five times for each sample on all analysis days. The filters were replaced after each sample in order to prevent contamination of cross-reactivity between samples. In order to produce repeatable values, it was necessary to "calibrate" the filters. This calibration involved wetting the filter with buffer and noting the filtration time of 2.0 mL of buffer both before and after each sample. The average buffer filtration time was then subtracted from the average time of the five runs for the blood sample. It should also be noted that after each run the filter was washed with buffer in order to minimize the amount of trapped debris.

**Electron Microscopy.** The erythrocytes were prepared by adding 1.0 mL of fixative containing equal volumes of phosphate buffered saline (PBS, pH 7.40) and Karnovsky’s fixative to 0.1 mL whole blood. The specimen was incubated at room temperature for 30 minutes after which it was centrifuged at 750 g for one minute. The packed cells were washed twice with PBS and then processed and examined by standard transmission electron microscopy (EM) procedures.†

**Hematologic Analyses.** Complete blood counts (erythrocytes, leukocytes, platelets) and differential leukocyte counts, as well as red cell indices, were determined for all samples on days 0, 7, 14, 15, and 16. The specimens were analyzed using a Technicon H-1 hematology analyzer according to the manufacturer’s procedure.‡

**REAGENTS/SOLUTIONS**

The phosphoric acid, thiobarbituric acid (TBA), TEP (1,1,3,3-tetraethoxypropane), methanol-NaOH solu-

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TABLE I

<table>
<thead>
<tr>
<th>Day</th>
<th>Controls μmol/L</th>
<th>Desferri-oxamine μmol/L</th>
<th>Statistical Significanceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.73 (0.18)</td>
<td>0.73 (0.18)</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>0.99 (0.19)</td>
<td>0.85 (0.11)</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>14</td>
<td>1.36 (0.38)</td>
<td>1.06 (0.23)</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>18</td>
<td>6.92 (2.11)</td>
<td>2.36 (0.44)</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation.
b Desferrioxamine-treated erythrocytes versus corresponding controls.

Statistical Analysis

Data computations included the means, standard deviations, Mann-Whitney and matched T-tests.

Results

Desferrioxamine (DM) is an effective antioxidant as shown in table I and figure 1, both of which demonstrate that the differences from the controls were significant on all days. This difference is particularly striking following incubation at 37°C (day 18), after which the red cells had been severely stressed, and there was increased hemolysis, breakdown of hemoglobin and liberation of iron. Table II and figure 2 also demonstrate that DM was effective in reducing erythrocyte deformability, especially after the first 7 days. After severe stress (day 18), the variation in filtration time of the controls was extensive with several exceeding 70 seconds; none of the DM-treated samples exceeded 40 seconds.

The EM studies showed increasing numbers of rounded, swollen erythrocytes, echinocytes (spiked red cells) and cellular debris with incubation time (figures 3 and 4). Compared with the controls, there were distinct trends for the...
EFFECT OF DESFERRIOXAMINE ON STORED ERYTHROCYTES

TABLE II

Erythrocyte Filtration Times Following Incubation with Desferrioxamine

<table>
<thead>
<tr>
<th>Day</th>
<th>Controls (Seconds)</th>
<th>Desferrioxamine (Seconds)</th>
<th>Statistical Significance^</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.9 (2.6)</td>
<td>4.9 (2.6)</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>14.9 (7.8)</td>
<td>10.1 (3.7)</td>
<td>P = 0.112</td>
</tr>
<tr>
<td>14</td>
<td>35.8 (4.7)</td>
<td>23.2 (5.7)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>18</td>
<td>47.4 (26.8)</td>
<td>30.3 (12.3)</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

^ Seconds

b Desferrioxamine-treated erythrocytes versus corresponding controls.

Discussion

Citrate-phosphate-dextrose (CPD), supplemented with adenine and dextrose, resulted in an increased storage time of whole blood from 21 to 35 days. More recently, other nutrient-anticoagulant solutions have been introduced. Solution AS-1, which consists of saline, adenine, dextrose, and mannitol, was shown to preserve adequately the erythrocyte viability up to 49 days. In addition, AS-3, which consists of added adenine, dextrose, sodium phosphate, chloride, and citrate, acceptably preserves stored red cells for 42 days. However, these nutrient-anticoagulant solutions require the initial removal of platelets, leukocytes, and plasma within 72 hours of phlebotomy, followed by the addition of the supplemented solutions, thereby adding significantly to the cost of blood processing; it also increases the possibility of bacterial contamination.

In addition to the positive effects of these additional nutrients, some of their success is probably due to the removal of neutrophils, which normally produce abundant free radicals and increase lipid peroxidation. Furthermore, AS-1 contains mannitol, an established free radi-

![Figure 2. Mean erythrocyte filtration times (seconds) compared with incubation time (days); controls vs. desferrioxamine (DM)-treated erythrocytes.](image-url)
FIGURE 4. Erythrocytes after 7 days with desferrioxamine additive, showing three spiked red cells (echinocytes) at bottom. There was a slight trend for more echinocytes in the untreated vs. the desferrioxamine-treated blood, but this difference was not statistically significant (magnification ×4875).
cal scavenger. In this regard, metal chelators bind iron, copper, and other transition metals, thereby decreasing their catalytic production of free radicals by the Fenton and/or Haber-Weiss reactions. Thus, our interest is to study the possibility of adding to the basic nutrient-anticoagulant solution (i.e., CPDA-1) safe antioxidants that might be effective in increasing the mean red cell life-span and hence the shelf-life of stored blood.

In the current study, DM was shown to be an excellent antioxidant by chelating iron, presumably released from the aging erythrocytes. Not only was DM effective in reducing lipid peroxidation (table I, figure 1), but it also decreased red cell filtration times (table II, figure 2) and thereby slowed the rate of erythrocyte deformability. With respect to the morphologic erythrocyte changes (figures 3 and 4), there appeared to be a distinct trend for the DM-treated red cells to retain their normal appearance longer than the corresponding control cells; however, this difference was not statistically significant. This was perhaps due to the difficulty not only of quantifying the number of rounded, swollen, and spiked cells but also to measure the degree of morphologic change. Future morphologic studies in this area may require not only a larger number of samples but possible changes in sample fixation and cell preparation techniques so that the morphologic changes are more consistent from field to field. In addition, identification of the earliest morphologic variations might be helpful in evaluating these qualitative changes.

In summary, DM is an effective antioxidant, not only with respect to decreasing red cell LP as measured by plasma MDA levels, but also in decreasing erythrocyte filtration rates, a measure of the preservation of erythrocyte shape, size, and deformability. These latter characteristics were only partially verified by the morphologic changes defined by electron microscopy.

References

16. Gutteridge JMC, Quinlan GT. Malondialdehyde formation from lipid peroxidation in


