Lipid Peroxidation Compared in Stored Whole Blood with Various Nutrient-anticoagulant Solutions*†

JOSEPH A. KNIGHT, M.D.,‡§
DOUGLAS A. SEARLES, B.S.,§
and ROBERT C. BLAYLOCK, M.D.§

Pathology and Laboratory Medicine Service,
Salt Lake VA Medical Center‡
and
Department of Pathology,
University of Utah School of Medicine,§
Salt Lake City, UT 84132

ABSTRACT

Since the introduction of acid-citrate-dextrose (ACD) in 1947 to anticoagulate and preserve whole blood for transfusion, various improved formulas, such as citrate-phosphate-dextrose-adenine (CPDA-1), have been successfully introduced, extending the current acceptable blood storage time to 35 days. Additional nutrient-anticoagulant solutions, including CPDA-2, CPDA-3, AS-1, and AS-5, among others, have more recently been studied with comparable results. In this study, lipid peroxidation (LP) was compared, as determined by the measurement of plasma malondialdehyde (MDA) by liquid chromatography, in stored blood preserved with various nutrient solutions with blood treated with CPDA-1. Although minor but inconsistent differences were noted with most of these solutions compared with CPDA-1, AS-1 gave consistently lower MDA levels (P < 0.01). Further improvements in red cell storage and viability may require approaches other than nutrient formula variations, such as the addition of metal chelating agents or other selected antioxidants to the nutrient-anticoagulant solutions, donor supplementation with vitamin free radical scavengers, or other as yet undetermined conditions.

Introduction

The nutrient-anticoagulant solution acid-citrate-dextrose (ACD), introduced in 1947, improved the preservation of whole blood for transfusion therapy. Since then, various improved nutrient-preservative formulas have been proposed to increase the viability and storage time of whole blood preparations. Thus, in 1957 Gibson et al reported that blood collected and stored in citrate-phosphate-dextrose (CPD) was more stable than that collected and stored in ACD, a finding later supported by oth-
Subsequent studies reported on the successful use of CPD supplemented with 0.25 mM adenine. Supplementation with citrate-phosphate-dextrose-adenine solution with additional added glucose (CPDA-1) has been the primary nutrient anticoagulant solution since 1978. In the process, the acceptable storage time was increased from 21 to 35 days. In this regard, Moroff et al reported improved maintenance of red cell adenosine triphosphate (ATP) levels in CPDA-1 in comparison with CPD, although several other measured parameters were similar. More recently, other nutrient solutions have been proposed for whole blood and red cell concentrate storage; these include CPDA-2, CPDA-3, AS-1, AS-3, and AS-5.

In addition to the significantly improved erythrocyte survival by the use of certain additive nutrients, others have examined the effects of various antioxidants on stored plasma and erythrocytes. Thus, Lee reported that the addition of reduced glutathione (GSH) and ethylenediaminetetraacetic acid (EDTA) significantly decreased lipid peroxidation (LP) in plasma stored at 4 to 5°C. When both were present, their antioxidant effects were additive. In addition, Lachant et al noted increasing Heinz body formation and decreased glutathione levels in stored red cells. More recently, significantly reduced LP was reported by us in stored whole blood following the addition of various metal-binding chelators to both non-irradiated and irradiated blood. Furthermore, donor supplementation with vitamins C and E, well-known free radical scavengers, resulted in decreased LP in both irradiated and non-irradiated stored whole blood. As an additional suggested parameter for comparing red cell viability in various nutrient-preservation solutions, this current study compares LP in CPDA-1 preserved whole blood with blood stored in CPDA-2, CPDA-3, AS-1, and AS-5. This comparison was made by measuring plasma malondialdehyde (MDA) at varying storage times. The plasma potassium levels were also quantified at the same time intervals and estimated the degree of plasma hemolysis.

Materials and Methods

Specimens and Procedures

Citrate-phosphate-dextrose-adenine is currently recommended as the nutrient-anticoagulant solution for blood storage. Therefore, in this study other preservative solutions were compared with it. Thus, duplicate 7.0 mL blood samples were anticoagulated with CPDA-1* in the same ratio as present in the normal donor containers (0.8 mL CPDA-1 solution added to 7.0 mL of whole blood) and stored at 3°C until baseline analysis for malondialdehyde and potassium (K+), which were completed within 12 hours. The CPDA-2 and CPDA-3 solutions were prepared from CPDA-1 containers as follows.

A sampling site was placed into two CPDA-1 bags. Adenine plus dextrose mixtures were prepared in two separate test tubes. To the first tube (CPDA-2) were added 17.3 mg adenine and 0.8 g dextrose; to the second tube (CPDA-3) were added 17.3 mg adenine and 1.2 g dextrose. Four mL of solution were then removed from the CPDA-1 container bag and added to each test tube, mixed well until dissolution, and then reintroduced into the corresponding CPDA-1 bags, thereby producing CPDA-2 and CPDA-3 nutrient solutions. This procedure was twice repeated to ensure complete transfer of the adenine and glucose to their

---

* Baxter Healthcare Corp., Fenwal Division, Deerfield, IL 60015.
† Sigma Chemical Co., St. Louis, MO 63178.
corresponding bags. In both cases, the total anticoagulant volume was 63 mL. Into duplicate 10 mL test tubes, 0.8 mL of the freshly prepared CPDA-2 and CPDA-3 solutions was transferred and 7.0 mL of freshly drawn blood were added for plasma MDA and K$^+$ analysis and subsequent storage.

The AS-1 (CPD-ADSOL)* triple bag collection system, with 100 mL of preservative fluid, contains 2.2 g dextrose, 900 mg NaCl, 750 mg mannitol, and 27 mg adenine. In the final AS-1 blood solutions, the same reagent ratios were used as previously described; that is, 0.8 mL of CPDA-1 was added to a 10 mL test tube containing 7.0 mL of whole blood obtained by venipuncture. The tubes were gently centrifuged at 500 rpm for five minutes and 1.36 mL of plasma was removed and replaced with 1.36 mL of the AS-1 preservative solution from the AS-1 bags. The tubes were then stored at 3°C and analyzed for MDA and K$^+$ on days 7, 14, and 18 as for all the other samples.

Each 63 mL of AS-5$ nutrient/anticoagulant solution in double blood bags contains 206 mg of citric acid (hydrous), 1.66 g of sodium citrate (hydrous), 140 mg of monobasic sodium phosphate, 1.83 g of dextrose, and 17.3 mg of adenine. To appropriate 10 mL test tubes containing 0.8 mL of the AS-5 solution were added 7.0 mL of blood obtained by venipuncture, gently mixed, stored as for the other nutrient-treated blood samples and analyzed for MDA and K$^+$ as described previously.

Plasma MDA levels were determined in duplicate by high performance liquid chromatography (HPLC) as previously reported with modifications with respect to the equipment used. Plasma K$^+$ was quantified using a discrete chemistry analyzer according to the manufacturer's procedure. For the baseline K$^+$ measurements (day 0), the serum/plasma procedure was followed; for subsequent measurements (days 7, 14, 18), the urine procedure was used.

All whole blood samples were stored at 3°C, mixed several times by gentle inversion every other day, including the days of MDA and K$^+$ analysis, and the plasma analyzed following gentle centrifugation at approximately 75 g for five minutes on days 7 and 14. The samples were then placed in a 37°C incubator to simulate in vivo conditions, again mixed several times by gentle inversion on day 16 and immediately before analysis on day 18. They were then centrifuged at about 2,500 g for five minutes and the plasma analyzed for MDA and K$^+$. In addition, visual plasma inspection for hemolysis was recorded on each analysis day.

REAGENTS/SOLUTIONS

The phosphoric acid, thiobarbituric acid (TBS), 1,1,3,3-tetraethoxypropane (TEP), methanol-NaOH solution, phosphate buffer, and mobile phase solutions were all purchased and prepared as previously reported. Reagents for the K$^+$ determinations were obtained from the chemistry analyzer manufacturer as noted previously.

STATISTICAL ANALYSIS

Owing to the consistency of the MDA and K$^+$ levels (MDA but not K$^+$ was analyzed in duplicate), seven samples were randomly chosen from each CPDA-1 control group (total 28 samples). These were compared with 11 samples from each of CPDA-2, CPDA-3, AS-1, and AS-5, all of which were also analyzed in duplicate.

---

† Terumo Medical Corp., Somerset, NJ 08873.

§ Astra-8, Beckman Instruments, Brea, CA 92621.
Data computations included means, standard deviations, and the Mann-Whitney test.

Results

The results comparing plasma MDA levels from CPDA-1 at 7, 14, and 18 days with those obtained from blood anticoagulated and preserved with CPDA-2, CPDA-3, AS-1, and AS-5 are presented in table I. Although slight differences were sporadically noted between CPDA-1 and CPDA-2, CPDA-3, and AS-5, they were inconsistent. That is, with each nutrient solution there were slight but significant differences from CPDA-1 on one of the three days, but not on the other two. However, plasma MDA levels from blood anticoagulated with AS-1 were significantly lower than those from CPDA-1 on all three days (P < 0.01 or less).

The visual estimation of hemolysis was similar with all of the nutrient-anticoagulant solutions. There was no hemolysis on day 0, but it progressively increased on days 7, 14, and 18 averaging respectively, trace, 1+, and 3+. In this regard, plasma K\(^+\) levels in all cases progressively increased from a mean of about 4.4 mmol per L on day 0 to near 50 mmol per L on day 18 (table II). Again, slight but inconsistent differences were noted between CPDA-1 and the other preservative solutions, although K\(^+\) levels from AS-5 were significantly lower than CPDA-1 on days 7 and 18 (P < 0.05), but not on day 14.

Discussion

Over the past 50 years, various anticoagulant-nutrient solutions have been introduced to make blood more available, economical, and safer for transfusion by improving erythrocyte function and viability over a longer period of time. Thus, the introduction of ACD\(^4,17\) made it possible to store safely whole blood at 3 to 4°C for 21 days. This nutrient-anticoagulant solution was subsequently replaced by the introduction of CPD, which was further improved by the addition of adenine. This latter solution, plus additional glucose (CPDA-1), lengthened the acceptable storage time to 35 days.\(^2,3\) These additional additives maintain optimal blood concentrations of both 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP).\(^15\) With this success, increased levels of adenine (CPDA-2) or adenine plus glucose (CPDA-3), as well as other variations led to still other preservative solutions, including AS-1, AS-3, and AS-5.\(^1,6,18\)

Recent studies have compared these various preservative solutions in both

<table>
<thead>
<tr>
<th>Day</th>
<th>CPDA-1</th>
<th>CPDA-2</th>
<th>CPDA-3</th>
<th>AS-1</th>
<th>AS-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.16 (0.23)</td>
<td>1.16 (0.23)</td>
<td>1.16 (0.23)</td>
<td>1.16 (0.23)</td>
<td>1.16 (0.23)</td>
</tr>
<tr>
<td>7</td>
<td>1.69 (0.53)</td>
<td>1.37 (0.17)(^a)</td>
<td>1.73 (0.46)(\text{ns})</td>
<td>0.98 (0.23)(^c)</td>
<td>1.88 (0.47)(\text{ns})</td>
</tr>
<tr>
<td>14</td>
<td>2.26 (0.39)</td>
<td>2.43 (0.38)(\text{ns})</td>
<td>2.55 (0.44)(^a)</td>
<td>1.80 (0.36)(^b)</td>
<td>2.49 (0.53)(\text{ns})</td>
</tr>
<tr>
<td>18</td>
<td>6.45 (1.21)</td>
<td>6.05 (1.60)(\text{ns})</td>
<td>6.85 (1.03)(\text{ns})</td>
<td>5.05 (1.20)(^b)</td>
<td>5.55 (0.88)(^a)</td>
</tr>
</tbody>
</table>

* \(\mu\)mol per L

\(^a\) Mean ± Standard Deviation

Statistical significance, levels of plasma malondialdehyde in nutrient/anticoagulant solutions compared with CPDA-1: ns = not significant; \(a = P < 0.05; b = P < 0.01; c = P < 0.001\)
TABLE II
Comparison of Plasma Potassium Levels* from Blood Stored In Citrate-Phosphate-Dextrose-Adenine (CPDA-1) versus Other Nutrient/Anticoagulant Solutions*

<table>
<thead>
<tr>
<th>Day</th>
<th>CPDA-1</th>
<th>CPDA-2</th>
<th>CPDA-3</th>
<th>AS-1</th>
<th>AS-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.4 (0.7)</td>
<td>4.4 (0.7)</td>
<td>4.4 (0.7)</td>
<td>4.4 (0.7)</td>
<td>4.4 (0.7)</td>
</tr>
<tr>
<td>7</td>
<td>22.1 (5.3)</td>
<td>20.3 (4.4) ns</td>
<td>20.0 (4.3) ns</td>
<td>22.7 (2.7) ns</td>
<td>17.5 (2.7) a</td>
</tr>
<tr>
<td>14</td>
<td>32.1 (4.9)</td>
<td>27.7 (5.0) b</td>
<td>26.0 (4.1) b</td>
<td>28.1 (4.6) a</td>
<td>32.1 (4.7) ns</td>
</tr>
<tr>
<td>18</td>
<td>50.2 (3.8)</td>
<td>48.9 (4.1) ns</td>
<td>48.4 (3.5) ns</td>
<td>49.9 (3.5) ns</td>
<td>47.4 (3.4) a</td>
</tr>
</tbody>
</table>

*mmol per L *Mean ± Standard Deviation
Statistical significance, potassium levels in nutrient/anticoagulant solutions compared with CPDA-1:
ns = not significant; a = P < 0.05; b = P < 0.01

irradiated and non-irradiated blood by measuring changes in glucose, 2,3-DPG, lactate dehydrogenase, K⁺, among other analytes. Thus, with respect to red cell K⁺ loss, AS-1 appeared to be superior to CPDA-1 in both irradiated and non-irradiated stored erythrocytes. These authors noted the possibility that the selected anticoagulant solution and storage time may be significant, at least in the transfusion of post-irradiated blood to neonates in comparison with adults. Others noted that after 35 days storage, the ATP level of CPDA-1 red cell concentrates had decreased significantly more than CPDA-2 or CPDA-3 treated red cells. Conversely, 2,3-DPG deteriorated faster during the first 10 days than in CPDA-2 and CPDA-3. Furthermore, the use of a commercially available rejuvenation solution was shown to be efficacious in raising ATP and 2,3-DPG levels in erythrocytes stored in AS-1 and AS-3 for 42 days. Nevertheless, few major blood storage improvements have been noted with these more recently introduced solutions.

These earlier studies suggested to us that other parameters should be studied in order to increase further our knowledge regarding red cell aging. Therefore, the present authors elected to compare LP, an autocatalytic free radical process, in red cells stored in CPDA-1 with CPDA-2, CPDA-3, AS-1, and AS-5 nutrient-anticoagulant solutions by measuring plasma MDA levels following storage at various times (7 and 14 days at 3°C and after four additional days at 37°C). In this regard, our data demonstrate a consistent and significant decrease in LP in blood stored in AS-1 compared with the other nutrient-anticoagulant solutions (table I). Importantly, this preservative solution contains mannitol, a well recognized free radical scavenger. On the other hand, although plasma K⁺ levels in blood stored in AS-1 were previously noted to be consistently less than with CPDA-1, our results were inconsistent in this regard (table II). However, AS-5 treated blood did have a significantly lower K⁺ on two of the three analysis days. In addition, although not an accurate evaluation, visual hemolysis was similar in all of the stored specimens regardless of the preservative solution.

Using plasma MDA as an indicator of the severity of LP, this study suggests that by changing the nutrient composition alone, there will be little further improvement in preserving stored blood. However, as shown in this study, the inclusion of the antioxidant mannitol in AS-1 was probably the significant difference that led to decreased LP. This is in agreement with our previous studies in which it was demonstrated by us that...
the addition of various metal chelating agents slowed the initiation step of LP by binding released iron, a potent catalyst for free radical formation, from the aging red cells. Furthermore, the addition of glutathione, an important naturally occurring antioxidant, also decreased LP in stored blood. In addition, donor supplementation with vitamins C and E, free radical scavengers in aqueous solutions and lipid cell membranes, respectively, resulted in significant LP reductions in stored red cells.

This and other reports suggest that further studies are in order to test red cell function, viability, and longevity using these and other possible modalities in order to improve the storage of whole blood for transfusion.

References