Effects of four additive solutions on canine leukoreduced red cell concentrate quality during storage

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Key Words
Blood, dog, erythrocyte, filter, transfusion

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Background: Additive solutions (AS) and prestorage leukoreduction (LR) are important tools used to maintain erythrocyte viability during storage and avoid transfusion reactions in recipients, respectively.

Objectives: The purpose of the study was to determine the efficacy of a WBC filter (Immugard IIIRC) and compare the effect of 4 AS (phosphate-adenine-glucose-guanosine-gluconate-mannitol [PAGGGM], saline-adenine-glucose-mannitol [SAGM], Adsol, Optisol) on the in vitro quality of canine leukoreduced packed RBC units (pRBC) stored for 41 days.

Methods: Five hundred milliliters of blood were collected from 8 healthy dogs each into 70 mL of citrate-phosphate-dextrose (CPD) solution, and were leukoreduced by a polyurethane filter. pRBC of each dog were divided equally into 4 bags containing a different AS. Bags were stored for 41 days at 4°C and evaluated every 10 days. Variables analyzed included pH, PCV, and% hemolysis, and lactate, glucose, potassium, sodium, ATP, and 2,3-diphosphoglycerate (2,3-DPG) concentrations.

Results: The LR resulted in residual WBC counts comparable to human standards. During storage, pH, and glucose, 2,3-DPG, and ATP concentrations decreased, and hemolysis, and lactate, sodium, and potassium concentrations increased (P < .05). Significant differences between AS were seen in the glucose and sodium concentrations, due to the composition of AS. Also, the pH maintained by PAGGGM at day 21 was significantly higher than that seen with SAGM or Adsol.

Conclusions: All AS used gave satisfactory results during the first 21 days of storage based on the degree of hemolysis, and on ATP and 2,3-DPG concentrations. When compared with day 1 values, significant changes were seen in these variables by day 31 with all AS.

Introduction

When blood is stored for transfusion purposes, biochemical and morphologic changes, also known as the “storage lesion,” occur and can contribute to morbidity and mortality of critical patients.¹⁻⁵ Over the last 4 decades, researchers have investigated various blood storage solutions and techniques that improve the quality of the stored blood and minimize the storage lesion in people and animals.⁶⁻¹⁶ Additive solutions (AS), which are protein-free solutions added to the pRBC following plasma removal, were developed to prolong the life of the RBC during storage; they continue to be improved to achieve better results.⁵⁻¹⁶

Leukoreduction (LR) has become another important technique to improve blood quality and to avoid adverse effects, such as inflammation, febrile and allergic responses, infectious diseases, and alloimmunization reactions in the recipient.¹⁷⁻²¹

The storage lesion of canine stored blood units is similar to that seen in human blood products.¹¹ RBC-related ATP and 2,3-diphosphoglycerate (2,3-DPG), and glucose concentrations decrease over time, while lactic and pyruvic acids accumulate, and pH decreases.
The change in ATP and 2,3-DPG concentrations is extremely important, as without them the RBC cannot complete its in vivo functions properly.\textsuperscript{11–14}

Recent human studies reported the maintenance of in vitro ATP and 2,3-DPG levels with a new additive solution, phosphate-adenine-glucose-guanosine-glucuronate-mannitol (PAGGGM) (Sanquin Blood Supply Foundation, Amsterdam, the Netherlands). Compared with commercial solutions such as saline-adenine-glucose-mannitol (SAGM) (Terumo Penpol Limited, Jawaharnagar, Gujarat, India), PAGGGM is more alkaline, and contains no chloride.\textsuperscript{15,16} LR and other prestorage techniques also can influence the RBC viability during storage, and may have an impact on the transfused patient.\textsuperscript{22–24} Studies on biochemical variables in stored LR pRBC are incomplete in the veterinary literature, with only changes in ATP concentrations previously documented.\textsuperscript{17} A significant reduction in markers of inflammation has also been noted in healthy dogs receiving autologous leukoreduced blood.\textsuperscript{19}

In Brazil, SAGM is the AS that is commercially available, and its effects on canine RBC concentrates, also called packed RBC (pRBC), have not been previously reported. Effects of the new solution PAGGGM and the AS-5 solution Optisol (Terumo Corporation, Tokyo, Japan) have also not been documented in canine units. The purpose of our study was therefore to evaluate the efficacy of a prestorage LR filter and the effect of 4 AS on hematologic and biochemical variables of canine leukoreduced pRBC stored for 41 days. The formulations of all solutions used in this study are given in Table 1.

### Materials and Methods

#### Animal selection

Eight large-breed dogs (body weight over 40 kg) with docile and calm temperament, including 4 sexually intact females and 4 sexually intact males, were selected with written consent from private owners. Results of a CBC, chemistry profile, infectious disease screen, and physical examination were normal for each dog before the procedure. All the animals were observed for at least 30 min and clinically reevaluated after the procedure to guarantee their safety before leaving the facility with the owner. This study was developed in compliance with the Standards of Animal Ethics and Welfare recommended by the National Council on the Control of Animal Experiments and was further approved by the Research Ethics Committee of Universidade Federal do Rio Grande do Sul (UFRGS) (n. 12482).

#### Blood collection

All dogs were physically restrained and not sedated for blood collection. Five hundred milliliters of blood were collected from each dog into a primary blood bag (triple bag system), containing 70 mL of CPD solution (Terumo Corporation) as the anticoagulant–preservative, and weighed before any other procedure. A small aliquot (approximately 5 mL) of blood was removed from the collection bag tubing using aseptic technique for prefiltration WBC and platelet count and PCV determination.

#### Filtration

Bags containing the collected whole blood were cooled to 20–24°C using a closed system containing a buta-1,4-diol cooling plate (Compocool WB, Fresenius HemoCare, Bad Homburg, Germany). The bags were then attached to the filter system using sterile technique (Terumo Sterile Connecting Device – TSCD-II, Terumo Medical Corporation, Somerset, NJ, USA) and filtered through the LR filter into a secondary bag by gravity after breakage of an integral canula above the filter. The LR filter used in this study (Terumo Immugard III-RC, Terumo Corporation) is a third-generation polyurethane filter with a neutral charge, manufactured to remove both WBCs and platelets. The filtration time was determined from the moment blood started to flow through the tubing until the cessation of blood flow. The blood lost in the filter and tubing was determined by weighing the full secondary bag. The pre- and postfiltration weights were converted to milliliters by dividing the gram weight by 1.06.\textsuperscript{17} A second aliquot of the LR whole blood was taken using aseptic technique via sample tubing attached to the bag for postfiltration WBC, platelet count, and PCV determination.

#### WBC and platelet counts

Before filtration, WBCs and platelets were counted using an automated hematology analyzer (ABX Micros Abc Vet, Horiba Medical, ABX Diagnostics, Montpellier, France). The residual WBC and platelet counts were counted on a Nageotte hemocytometer (LO-Laboroptik GmbH, Bad Homburg, Germany) using Türk’s solution (Newprov, Pinhais, PR, Brazil) and on a Neubauer Bright line hemocytometer (Boeco Germany, Hamburg, Germany) using 1% ammonium oxalate, respectively. The following formula was used to determine leukoreduction:
% platelet and WBC depletion  
\[ = \frac{\text{Prefiltration counts} - \text{Postfiltration counts}}{\text{Prefiltration WBC or platelet counts}} \times 100 \]

### Component preparation

Blood from the secondary bag (ie, LR whole blood) was processed into pRBC and plasma by centrifugation at 4,050 g for 6 min at 4°C (Legend-RT plus, Sorvall, Thermo Scientific, Asheville, NC, USA).\(^{13,14}\) After centrifugation, plasma was expressed (Plasma Separation Stand, Terumo Medical Corporation) from the primary bag into a transfer bag, separated, and stored at \(\leq 30^\circ\text{C}\) for one year. Each pRBC specimen was split equally (approximately 55 mL per bag) into 4 smaller pediatric transfer bags (capacity of 100 mL) (JP Indústria Farmacêutica S.A., Ribeirão Preto, SP, Brazil), and 4 different additive solutions were added to the RBC concentrate (25 mL per bag), respecting the proportion of the standard method for a normal unit. The pRBC and additive solution transfer procedures were performed using sterile technique (Terumo Sterile Connecting Device – TSCD-II, Terumo Medical Corporation). The solutions used were SAGM, Adsol (Fenwal, Inc., Lake Zurich, IL, USA), Optisol, and PAGGGM (for detailed composition, see Table 1). Each smaller pRBC unit was stored at 4°C for 41 days in a standard blood bank refrigerator in a horizontal position; the pRBC units were manually gently mixed for 1 min every 10 days prior to the sampling procedure.\(^ {25,26}\)

### In vitro analyses

Blood samples were drawn with an 11-mL syringe from each unopened bag using sterile technique through a blood sampling port (JP Indústria Farmacêutica S.A.) and a 16-Gauge needle on days 1, 11, 21, 31, and 41 after collection. Each bag was manually mixed for 1 min before taking the sample. Samples were divided into 3 parts. One 2-mL aliquot was used for pH determination right after sampling by a pH benchtop meter (Thermo Scientific, Beverly, MA, USA). A second 3-mL aliquot was used for PCV and total HGB determination. The third 6-mL aliquot was used for the determination of % hemolysis, and supernatant lactate, glucose, potassium, sodium concentrations, RBC-ATP and 2,3-DPG concentrations.

The PCV was measured manually by centrifugation for 5 min at 10,000 g (SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). The total HGB concentration was measured by a cyanmethemoglobin method (Labtest Diagnóstica S.A., Lagoa Santa, MG, Brazil). The sample was then centrifuged at 2,000 g for 10 min at 4°C (Legend-RT plus, Sorvall, Thermo Scientific). The supernatant was used for plasma HGB determination using a micromodification of the Drabkin hemoglobin assay.\(^ {27}\) Hemolysis was expressed as a percentage of total HGB present in pRBC after

### Table 1: Composition of additive solutions used for storage of canine pRBC in the study.

<table>
<thead>
<tr>
<th>Component</th>
<th>Vol./mg (mmol/L)</th>
<th>Vol./mg (mmol/L)</th>
<th>Vol./mg (mmol/L)</th>
<th>Vol./mg (mmol/L)</th>
<th>Vol./mg (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>180.2</td>
<td>141.82</td>
<td>255.56</td>
<td>120.99</td>
<td>900.0</td>
</tr>
<tr>
<td>Adenine</td>
<td>135.1</td>
<td>0</td>
<td>2</td>
<td>27</td>
<td>122.09</td>
</tr>
<tr>
<td>Guanosine</td>
<td>283.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>900.0</td>
</tr>
<tr>
<td>Monobasic Sodium Phosphate</td>
<td>120</td>
<td>18.52</td>
<td>222.22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium phosphate Dibasic</td>
<td>141.95</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mannitol</td>
<td>182.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>58.45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium Gluconate</td>
<td>218.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>294.1</td>
<td>89.59</td>
<td>2634.92</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>192.1</td>
<td>15.53</td>
<td>298.41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total Mass (grams)</td>
<td>5.71</td>
<td>3.88</td>
<td>2.33</td>
<td>2.32</td>
<td>3.06</td>
</tr>
</tbody>
</table>

correction for the PCV, as follows28,29:

\[
\% \text{ hemolysis} = \frac{(100 - \text{PCV}) \times \text{supernatant HGB} (\text{g/dL})}{\text{total HGB} (\text{g/dL})}
\]

The third part was centrifuged as described for HGB determination, and the supernatant was used to determine glucose, potassium, and sodium concentrations by a blood gas analyzer (I-Stat 1 and CG8+ cartridges, Abbott Point of Care Inc., Princeton, NJ, USA), and lactate concentrations by an enzymatic method (Labtest Diagnóstica S.A.). The RBCs were used to determine ATP and 2,3-DPG concentrations. Briefly, 1 mL of the pRBC of the sample was transferred to tubes containing 3 mL of ice-cold 8% trichloric acid (T6399, Sigma-Aldrich, St. Louis, MO, USA) and mixed thoroughly. The tubes were centrifuged as described previously, and supernatants were stored at -80°C until analysis. The ATP concentration of the acid-extracted RBC was determined by a luminescence ATP detection assay system (ATPLite, Perkin Elmer Inc., Waltham, MA, USA), and the 2,3-DPG concentration was determined by an enzymatic method (Cat. No. 10 148 334 001, Roche Diagnostics GmbH, Mannheim, Germany), both using a microplate reader (SpectraMax M5, Molecular Devices Inc., Sunnyvale, CA, USA).30 Both supernatant samples had to be neutralized before analysis due to the low pH of trichloric acid that could interfere with the 2,3-DPG and ATP method. Briefly, approximately 60 µL of 1 M sodium carbonate solution was added to 500 µL of supernatant of each sample to reach a neutral pH.

At the end of 41 days of storage, the pRBC units were sent to a microbiology laboratory for aerobic and anaerobic blood culture.

Statistics

Statistical analysis was performed using a statistical package software system (PASW Statistics 18, IBM Corporation, Somers, NY, USA). One-way ANOVA, followed by Bonferroni correction, and Tukey post hoc test were used to compare the solutions on each day of storage. A T-test was used to compare day 1 with the other days of each variable. The results were considered to be significant when P-values were < .05.

Results

Efficacy of the WBC filter

The filtration process took approximately 20 ± 6 min, and the entire process (collection through storage) took approximately 6 h. Mean reductions of 99.8% and 95.9% were obtained for WBCs and platelets, respectively, with the filter used. The volume of blood lost to the filter was 46 mL. The residual WBC count mean was 0.48 ± 0.09 × 10⁶ per unit of canine whole blood. The mean RBC recovery was 98.14%.

Differences between additive solutions over time

The PCV of all pRBC units complied with the American Association of Blood Banks standard for human RBC concentrates (≤ 80.0%).18 Biochemical changes that occurred with storage are listed in Table 2 and shown in Figure 1. A statistically significant decrease (P < .05) in pH was observed in all solutions; the pH maintained by PAGGGM was significantly higher than that seen with storage in either SAGM or Adsol. A statistically significant increase (P < .05) in % hemolysis and in lactate concentration over time was observed in all solutions, while glucose concentration decreased statistically significantly (P < .05) over time in all solutions, with the exception of Adsol, which consistently maintained a significantly higher glucose concentration when compared with the other AS, due to its formulation (Figure 1).

The ATP and 2,3-DPG concentrations were statistically significantly decreased (P < .05) in all solutions over time (Figure 1). The solution PAGGGM maintained the highest concentrations of ATP and 2,3-DPG at 21 days, but the change was not statistically significant. In contrast, sodium concentrations were statistically significantly increased (P < .05) over time in all solutions, except with PAGGGM. The sodium concentration in the PAGGGM stored product was significantly different from the concentration of all other solutions, at any time period. Potassium concentrations were statistically significantly increased (P < .05) over time in all solutions.

Microbiologic cultures from all bags were negative.

Discussion

Biochemical changes occurring during storage of non-LR canine pRBC in specific AS or anticoagulant-preservation have been previously reported, and pRBC stored in the AS Adsol and Nutricel (Pall Corporation, East Hills, NY, USA) have shown superior in vitro and in vivo results when compared with the anticoagulant-preservation citrate-phosphate-dextrose-adrenaline (CPDA-1) solution.12-14 However, some countries, such as Brazil, lack commercial access to the
above AS, prompting the current study using other, more available solutions. Only limited studies on storage of LR canine pRBC and its effects on the blood product quality and on the recipient have been reported, thus further emphasizing the need for this study. 17,19–21

Leukoreduction has been associated with reduced mortality in specific human patient populations, due to avoidance of some transfusion reactions and better quality of pRBC units.21,31,32 Many countries have adopted universal LR. LR filters have some disadvantages, such as higher cost; according to a recent study, each filter can add $20–$65 to the cost of a human pRBC unit depending on the country.33 The cost-effectiveness and the quality of leukoreduced blood products are a debated issue nowadays with many studies reporting that the higher costs can be justified by the reduction of hospital charges and shortened hospital length of stay.33,34 The use of LR filters can lead to 10% of RBCs lost due to trapping in the fil-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 1</th>
<th>Day 11</th>
<th>Day 21</th>
<th>Day 31</th>
<th>Day 41</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAGM</td>
<td>7.27 (0.09)</td>
<td>7.33 (0.05)</td>
<td>7.08* (0.06)</td>
<td>7.02 (0.07)</td>
<td>7.03± (0.07)</td>
</tr>
<tr>
<td>Adsol</td>
<td>7.28 (0.04)</td>
<td>7.31 (0.09)</td>
<td>7.10* (0.07)</td>
<td>6.99 (0.09)</td>
<td>7.00* (0.09)</td>
</tr>
<tr>
<td>Optisol</td>
<td>7.30 (0.04)</td>
<td>7.34 (0.08)</td>
<td>7.15± (0.06)</td>
<td>7.05 (0.05)</td>
<td>7.05± (0.08)</td>
</tr>
<tr>
<td>PAGGGGM</td>
<td>7.22 (0.06)</td>
<td>7.39 (0.06)</td>
<td>7.19± (0.06)</td>
<td>7.07 (0.08)</td>
<td>7.13± (0.08)</td>
</tr>
<tr>
<td>Hemolysis (%)</td>
<td>0.30 (0.09)</td>
<td>0.64 (0.21)</td>
<td>0.67 (0.24)</td>
<td>1.13 (0.51)</td>
<td>1.31 (0.56)</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAGM</td>
<td>6.06 (1.69)</td>
<td>13.71 (2.22)</td>
<td>22.49 (3.39)</td>
<td>26.09 (3.55)</td>
<td>26.98 (2.14)</td>
</tr>
<tr>
<td>Adsol</td>
<td>6.22 (2.03)</td>
<td>13.63 (1.28)</td>
<td>20.47 (2.20)</td>
<td>27.21 (4.12)</td>
<td>29.45 (5.08)</td>
</tr>
<tr>
<td>Optisol</td>
<td>6.26 (1.25)</td>
<td>12.75 (1.66)</td>
<td>20.74 (1.75)</td>
<td>23.80 (2.85)</td>
<td>25.70 (1.68)</td>
</tr>
<tr>
<td>PAGGGGM</td>
<td>6.50 (1.63)</td>
<td>13.57 (2.48)</td>
<td>20.60 (2.35)</td>
<td>26.27 (3.67)</td>
<td>28.43 (5.67)</td>
</tr>
<tr>
<td>ATP (µmol/g Hb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAGM</td>
<td>3.08 (0.84)</td>
<td>3.03 (1.30)</td>
<td>2.00 (0.57)</td>
<td>1.02 (0.65)</td>
<td>0.50 (0.41)</td>
</tr>
<tr>
<td>Adsol</td>
<td>3.26 (0.84)</td>
<td>3.62 (2.07)</td>
<td>1.93 (0.51)</td>
<td>1.19 (0.62)</td>
<td>0.53 (0.50)</td>
</tr>
<tr>
<td>Optisol</td>
<td>2.45 (1.06)</td>
<td>2.77 (1.45)</td>
<td>1.96 (0.45)</td>
<td>0.73 (0.50)</td>
<td>0.29 (0.35)</td>
</tr>
<tr>
<td>PAGGGGM</td>
<td>3.31 (1.55)</td>
<td>4.06 (2.28)</td>
<td>2.42 (0.93)</td>
<td>0.92 (0.76)</td>
<td>0.35 (0.30)</td>
</tr>
<tr>
<td>2.3-DPG (µmol/g Hb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAGM</td>
<td>19.83 (2.38)</td>
<td>16.99 (4.96)</td>
<td>13.74 (4.19)</td>
<td>8.43 (1.52)</td>
<td>4.25 (2.21)</td>
</tr>
<tr>
<td>Adsol</td>
<td>20.00 (4.20)</td>
<td>19.01 (2.94)</td>
<td>16.74 (4.66)</td>
<td>10.27 (3.06)</td>
<td>6.21 (2.83)</td>
</tr>
<tr>
<td>Optisol</td>
<td>16.84 (4.46)</td>
<td>16.72 (4.26)</td>
<td>13.33 (3.49)</td>
<td>6.99 (2.36)</td>
<td>3.66 (1.66)</td>
</tr>
<tr>
<td>PAGGGGM</td>
<td>18.00 (4.06)</td>
<td>17.04 (4.67)</td>
<td>15.97 (4.66)</td>
<td>10.11 (3.93)</td>
<td>7.77 (4.05)</td>
</tr>
<tr>
<td>Na+ (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAGM</td>
<td>144.63* (2.20)</td>
<td>148.88* (1.55)</td>
<td>145.88* (1.07)</td>
<td>150.00± (0.53)</td>
<td>150.50± (0.76)</td>
</tr>
<tr>
<td>Adsol</td>
<td>143.13* (2.80)</td>
<td>152.38± (1.92)</td>
<td>153.00± (1.07)</td>
<td>153.38± (1.19)</td>
<td>153.63± (0.74)</td>
</tr>
<tr>
<td>Optisol</td>
<td>145.38± (1.85)</td>
<td>150.38± (1.92)</td>
<td>147.25± (12.98)</td>
<td>150.50* (0.53)</td>
<td>151.13* (0.83)</td>
</tr>
<tr>
<td>PAGGGGM</td>
<td>115.00± (9.02)</td>
<td>114.57± (4.31)</td>
<td>121.71± (12.11)</td>
<td>118.00± (1.15)</td>
<td>119.29± (1.80)</td>
</tr>
<tr>
<td>K+ (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAGM</td>
<td>3.13 (1.01)</td>
<td>4.45 (0.73)</td>
<td>3.96 (0.37)</td>
<td>4.09 (0.35)</td>
<td>4.15 (0.36)</td>
</tr>
<tr>
<td>Adsol</td>
<td>3.04 (0.83)</td>
<td>4.23 (0.61)</td>
<td>4.00 (0.39)</td>
<td>4.09 (0.38)</td>
<td>4.13 (0.42)</td>
</tr>
<tr>
<td>Optisol</td>
<td>3.16 (0.94)</td>
<td>4.26 (0.68)</td>
<td>4.05 (0.50)</td>
<td>4.16 (0.47)</td>
<td>4.20 (0.48)</td>
</tr>
<tr>
<td>PAGGGGM</td>
<td>2.79 (0.96)</td>
<td>3.74 (0.48)</td>
<td>3.80 (0.33)</td>
<td>3.81 (0.35)</td>
<td>3.90 (0.37)</td>
</tr>
</tbody>
</table>

DPG indicates 2,3-diphosphoglycerate; PAGGGGM, phosphate-adenine-glucose-guanosine-glucosamine-mannitol; SAGM, saline-adenine-glucose-mannitol. Data are means (SD). Superscript letters in the same column represent statistically significant differences (P < .05) between solutions at the same time.
ter, but prestorage LR also effectively removes WBCs and more than 90% of platelets before they can contribute to the storage lesion of RBCs or transfusion reactions mediated by platelets and RBCs. The LR filter used in this study showed a lower efficacy for WBC removal than that presented for human RBC concentrates in other studies. The time of filtration was longer and volume of blood lost to the filter was lower when compared with one other study in dogs, while a more recent study showed similar results. Although the filter efficacy was < 99.9%, the filtration process still met the AABB threshold of...
< 5 x 10^6 residual WBCs per unit, and the European requirements for WBC counts of < 1 x 10^6 WBCs per unit.

Four different AS were used in this study. The original AS were formulated to dilute the RBC with saline prior to transfusion, and in addition supplied glucose and adenine to maintain energy metabolism. Later studies resulted in the addition of mannitol to decrease hemolysis of the stored cells. The newer AS PAGGGM contains guanosine for improved maintenance of ATP, 2,3 DPG, and phosphate concentrations, the latter of which may act as buffers or can be used in the formation of organic phosphates.15,36,37

All variables analyzed in all of the AS showed time-dependent changes. Hemolysis was higher than anticipated in the older units, as the limits of hemolysis for human RBC concentrates are 0.8% and 1.0% in Europe and the USA, respectively.18 The increased hemolysis seen in the bags stored for 31 and 41 days may be related to the smaller volume of pRBC remaining in the bags at these time points, as previously noted in human blood.37 Statistically significant differences between solutions at all time points were identified only for glucose (higher glucose concentrations in Adsol) and sodium (lower sodium concentrations in PAGGGM), both a result of the formulation of the AS themselves. The formulation of Adsol contains more than twice the amount of glucose (dextrose) compared with the other additives used in the study, contributing to the increased residual glucose concentration over time. High glucose levels can lead to an increase in insulin in the transfused individual38, which should be taken into consideration if a large volume of Adsol containing pRBC is needed and if the recipient suffers metabolic dysfunction.39

The lower sodium seen in PAGGGM is also a result of the formulation of this AS. The composition of PAGGGM was originally based on the hypothesis of Meryman and Hornblower that a chloride-free AS would aid in the maintenance of RBC-related 2,3 DPG and ATP concentrations throughout the storage period.40 Saline is thus replaced in this additive with a lower concentration of sodium as sodium gluconate used instead. It is unlikely that the low sodium concentration in PAGGGM would have any deleterious effect on the recipient. PAGGGM is also a more alkaline solution than other AS, with a higher pH, normally favoring formation of 2,3 DPG. In the present study, however, a statistically significant pH change was only seen at day 21, when compared with the other AS.

In the current study, there were no statistically significant difference between the ATP and 2,3-DPG concentrations, but ATP concentrations were transiently higher than those reported in previous studies on canine units11–14,17, which was different from a previous study on leukoreduced canine units.17 Recent studies have shown the influence of blood storage and concentrations of 2,3-DPG and ATP on the clinical outcome of patients who require blood transfusion.1–5 Maintenance of ATP and 2,3-DPG concentrations is a desirable characteristic, as ATP is needed to maintain RBC function and integrity; and 2,3-DPG improves the oxygen release to tissues.1–9 Low concentrations of ATP can lead to deformability and exposure of the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine, which are normally concentrated on the inner surface of the RBC membrane and are one of the signals for their removal from the circulation. People with ATP levels < 4 μmol per g HGB risk a higher incidence for PS exposure.15 The minimum recommended range of ATP concentration for stored pRBC is between 2.3 and 2.7 μmol/g HGB, which has been correlated with 75% of transfused RBCs remaining in circulation for 24 h posttransfusion in people.41 A significant association was found between RBC deformability and ATP concentrations, ATP concentrations < 2.5 μmol/g HGB.42 Concentrations of ATP < 0.75 μmol/g HGB are believed to be a limiting factor in RBC viability in dogs; therefore, storage of LR canine pRBC to day 41 would not be acceptable, as indicated in the present study.43 Concentrations of 2,3-DPG were > 11 μmol per g HGB until day 21 in all solutions, which is close to the values found in the blood of healthy and anemic dogs.44 PAGGGM and Adsol units appeared to have higher concentrations of 2,3-DPG at days 31 and 41 when compared with the other AS, but the differences were not statistically significant. The decrease in the concentrations of 2,3-DPG during storage was similar to that reported in previous human and canine studies for ADSOL and SAGM solutions11–14,17, but was lower than results previously reported for PAGGGM units in people.16 As 2,3-DPG binds to the β-subunits of HGB, shifting the oxygen dissociation curve to the right and allowing enhanced release of oxygen, its depletion during RBC storage is postulated to decrease microcirculatory oxygen release. Higher 2,3-DPG concentrations are a desirable characteristic as the de novo synthesis of 2,3-DPG can take up to 24 h to reach normal levels and, although unlikely, it can affect critical patients with a precarious oxygen balance.14,45

Besides the in vitro biochemical alterations, RBC storage lesions can result in immunomodulatory and inflammatory consequences in transfusion recipients.19,21,22 The clinical impact of pRBC storage time is an important and controversial issue, and there are several large randomized human clinical
trials currently underway that are investigating this issue. 41

In the present study, satisfactory results were obtained with all AS during the first 21 days of storage when compared with other canine pRBC studies. 12,13,17,19 Further evaluation of the AS used in this study is warranted, using in vivo analysis to determine posttransfusion stability and viability. Nonleukoreduced units could also be evaluated to further test these storage solutions in dogs.

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