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ORIGINAL RESEARCH

Comparison of equine platelet function and survival in whole blood collected in acid-citrate-dextrose solution or citrate-phosphate-dextrose-adenine solution

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Background: Equine whole blood collection and storage methods have been evaluated to assess red blood cell viability; however, platelet (PLT) viability has not been comprehensively assessed.

Objectives: The purpose of the study was to compare viability of PLTs collected in whole blood into 2 different anticoagulants.

Methods: Whole blood from 6 healthy adult Thoroughbred horses was collected into citrate-phosphate-dextrose-adenine (CPDA) or acid-citrate-dextrose (ACD). Platelet count, pH, and concentrations of glucose, lactate, carbon dioxide, oxygen, bicarbonate, sodium, potassium, and chloride were measured within 10 minutes of collection and then again one hour later at which time PLT aggregometry was performed to assess PLT function.

Results: Aggregometry mean amplitudes were significantly higher in CPDA compared to ACD. Blood glucose, pH, bicarbonate, sodium, and lactate concentrations were significantly higher in CPDA compared to ACD. Lactate concentration was higher following one hour in either anticoagulant. Potassium, oxygen, and carbon dioxide concentrations were significantly higher in ACD compared to CPDA at collection.

Conclusions: Platelet aggregometry results suggest that CPDA is superior to ACD for maintaining PLT viability following whole blood collection. This may be associated with the higher, more neutral pH as well as an increase in glucose available for metabolism. Although lactate was increased in the CPDA samples it was not high enough to decrease pH and therefore may not have been high enough to cause morphologic lesions and loss of PLT viability.

Introduction

Equine blood products are being more commonly used as part of many therapeutic protocols including treatment of anemia, coagulopathies, musculoskeletal diseases and lacerations.[1–5] The first step in the manufacturing protocols of any of these products is whole blood collection. Equine whole blood collection and storage methods have previously been evaluated to assess RBC viability[6]; however, platelet (PLT) viability has never been comprehensively assessed.[7] Recent studies evaluated effects of different anticoagulants on PLT counts and growth factor release in equine pure platelet-rich plasma (PRP) and pure PLT-rich gel, but did not evaluate citrate-phosphate-dextrose-adenosine (CPDA), a frequently used anticoagulant and preservative for whole blood collection in veterinary species.

Acid-citrate-dextrose (ACD) solution was the first anticoagulant and preservative used most commonly for storage of human whole blood. Citrate-phosphate-dextrose (CPD) was then introduced, resulting in improved preservation of 2,3-diphosphoglycerate concentration. The addition of adenine produced the formulation CPD with adenine (CPDA), resulting in higher ATP concentrations in stored blood as well as prolonged RBC storage life.[8] Acid-citrate-dextrose

solutions have now largely been replaced by CPD and other additive solutions for human, canine, and feline whole blood storage. Studies on the posttransfusion viability of canine and feline RBC have shown that whole blood can be stored with satisfactory posttransfusion viability for up to 35 days in CPDA solution, compared with 21 days for blood stored in ACD solution.[9]

Platelets are reactive cells that can be irreversibly activated during processing and storage.[10] Platelet activation, and subsequent cell damage, can be induced by shear stress[11], contact with charged surfaces, changes in temperature[12], and decreased oxygen availability. It is therefore important to ascertain stable and reproducible blood collection standards to maintain high viability of these cells.

We hypothesized that viability of equine PLTs will be improved with collection in CPDA compared to ACD. The aim of the study was to establish which anticoagulant is preferable for collection of whole blood for optimum PLT viability. Assays were conducted after collection and one hour postcollection to determine potential optimization of the clinical transfusion practices in our teaching hospital.

Materials and Methods

Horses

Blood was obtained from 6 adult Thoroughbred geldings, ages ranging from 10 to 24 years (mean 16 years old) from the research herd. They were determined to be healthy based on physical examination, a CBC, and serum biochemical evaluation. Horses were excluded from the study if they had received any anti-inflammatory medications for 2 weeks prior to the study. The experimental protocol was approved by the institutional animal care and use committee of the University of California–Davis (protocol # 17892).

Blood collection

Prior to blood collection, an area over the jugular vein was clipped and prepared following standard aseptic protocols (chlorhexidine and alcohol). Sixty mL of blood were collected by gravity flow using a 19-gauge butterfly needle connected to a 60 mL syringe (Monoject 60 cc Luerlock; Covidien LLC, Mansfield, MA, USA) containing 7 mL of either ACD or CPDA and was transferred to a 60 mL blood collection bag (Animal Blood Resources International, Stockbridge, MI, USA) via a 3-way stop cock (Smiths Medical ASD Inc., Dublin, OH, USA).

Blood storage and sampling

Blood bags containing whole blood were kept at room temperature, and each bag was tested for PLT count, pH, and concentrations of glucose, lactate, carbon dioxide, oxygen, bicarbonate, sodium, potassium, and chloride within 10 min of collection, and then again one h later at which time PLT aggregometry also was performed.

Platelet count

Platelet counts were performed on all samples at the time of collection and one h later, using the Siemens Advia 120 Hematology System (Siemens Healthcare Solutions, Erlangen, Germany). The presence of PLT clumps was also microscopically evaluated.

Blood gas analysis

Blood gas analysis was performed on all samples at the time of collection and one h later using the ABL81500 Flex Blood Gas Analyzer (Radiometer Medical ApS, Brønshøj, Denmark). Briefly, 100 μL of anticoagulated whole blood (room temperature) were collected into plain glass hematocrit tubes and placed into the sample port. Samples were then aspirated and automated analysis was performed.

Platelet aggregometry

Red blood cells were allowed to settle out of whole blood, yielding a supernatant of PRP. The PRP from each sample was decanted into a 1.5 mL conical tube, and, depending on the PLT count, samples were diluted in PLT-poor plasma to achieve a final concentration of approximately 250,000 PLTs/μL (±80,000). Aggregometry was performed with a temperature-controlled aggregometer (Chronolog Corporation Optical Aggregometer model 490, Havertown, PA, USA). A zero baseline of resting (unstimulated) PLTs was established. Platelets were activated with human γ-thrombin (Thrombin; Sigma Chemical Co., St. Louis, MO, USA; final concentration 0.5 U/mL). Manufacturer's software (Chronolog Corporation) was used to calculate amplitude and slope.

Statistical analysis

Normality of the data could not be evaluated due to the small sample size. Data were reported as means or medians. Aggregometry results were compared

using a paired *t*-test. The pH at time zero and at one h was compared between anticoagulants using the Wilcoxon signed rank test. Repeated measures one-way ANOVA was used to compare blood gas analysis results and PLT counts at time zero and one h following collection. Post hoc analysis of the means between the 2 groups was performed using the Tukey's test. Statistical analysis was performed using a commercial software program (Graph Pad Prism 6, La Jolla, CA, USA). In all cases, $P \leq .05$ was considered significant.

Results

Platelet counts

The mean PLT counts in the group of 6 healthy horses prior to blood collection were comparable and within the RI. There was no significant difference in PLT counts between the different anticoagulants and at collection (ACD 88,666/µL ± 48,285/µL; CPDA 87,666/µL ± 46,919/µL) compared to one hour later (ACD 104,333/µL ± 28,175/µL; CPDA 98,500/µL ± 25,789/µL). Platelet clumping was noted in some time zero samples and a majority of the samples at time one hour (Table 1).

Platelet aggregometry

Platelet-rich plasma from one horse was excluded from the aggregometry study as it was unresponsive to thrombin activation. The area under the curve, lag times, and slopes were not significantly different between the 2 anticoagulant groups. The amplitudes were significantly different ($P < .05$), with greater mean amplitude noted in CPDA (mean 22.80 ± 2.280) vs ACD (mean 18.60 ± 2.191) indicating a stronger response to thrombin activation when blood was stored in CPDA.

Table 1. Degree of platelet clumping in healthy equine whole blood preserved/stored in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose-adenosine (CPDA) for one hour. A small number of samples (2 of 6) showed platelet clumping (+) at time 0, while 3 of the 6 samples showed clumping at one hour after collection (Time 1).

	Time 0 ACD	Time 0 CPDA	Time 1 ACD	Time 1 CPDA
Horse 1	−	−	−	−
Horse 2	−	−	+	+
Horse 3	−	−	+	−
Horse 4	+	+	−	+
Horse 5	−	+	+	+
Horse 6	+	+	+	+

Blood gas analysis

The pH was significantly lower in PLT stored in ACD compared to CPDA at the time of collection and one hour postcollection ($P = .03$ for both). For each anticoagulant, there was no significant difference between pH at collection and after one hour (Figure 1A). There
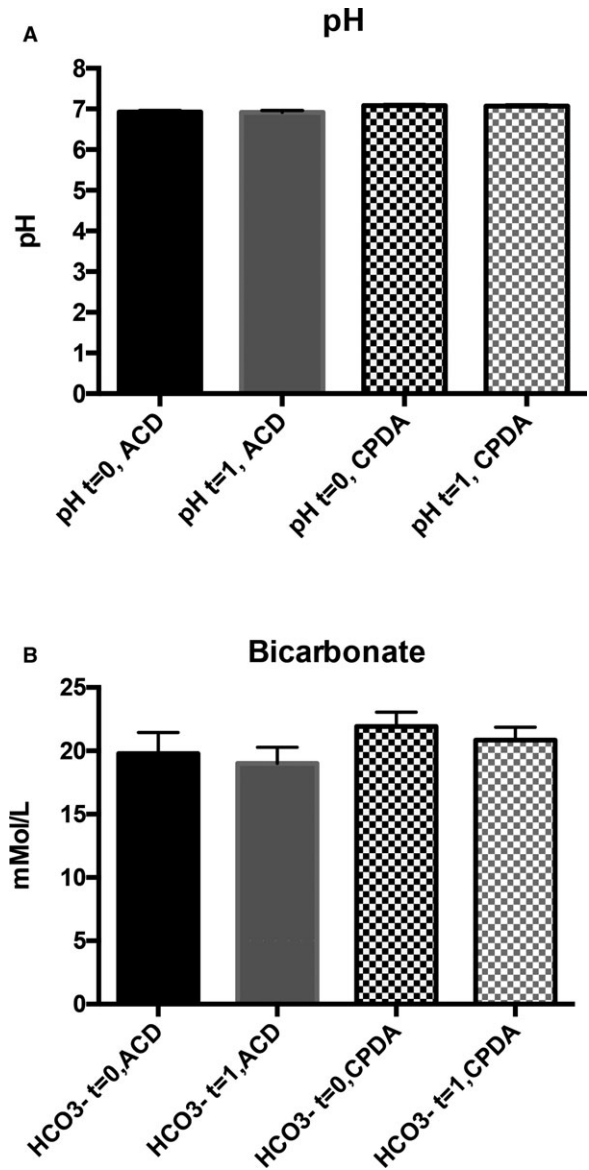
Figure 1. Means of pH (**A**) and bicarbonate concentration (**B**) measured in healthy equine blood preserved/stored in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose-adenosine (CPDA) for one hour ($t = 1$). (**A**) The pH was significantly lower in ACD compared to CPDA at the time of collection ($t = 0$) and one hour later. The pH at the time of blood collection was not significantly different at one hour for blood collected in ACD or CPDA. (**B**) Bicarbonate concentration (mM/L) was significantly lower in ACD compared to CPDA at the time of blood collection. It was also significantly lower in CPDA and ACD after one hour. *$P < .05$; **$P < .01$.

was no significant difference in chloride concentration in any of the groups or at any time point. Bicarbonate concentration was significantly lower following one hour in either anticoagulant (ACD $P < .05$; CPDA $P < .01$), and significantly lower in ACD compared to CPDA at both time points ($P < .01$ for both) (Figure 1B).

Lactate concentrations were significantly higher in bags with CPDA compared to ACD at the time of collection ($P < .001$) and at one hour postcollection ($P < .01$). Lactate concentrations were also significantly higher in both anticoagulants at one hour postcollection compared to collection ($P < .01$ for both) (Figure 2A). Glucose concentration was significantly higher in bags with CPDA compared to ACD at collection ($P < .001$) and one hour postcollection ($P < .01$). Glucose was also significantly higher after one hour in ACD ($P < .01$) but no significant difference was noted after one hour in CPDA (Figure 2B).

Sodium concentration was significantly higher in bags with CPDA compared to ACD at both time points ($P < .001$ for both), and significantly higher after one hour in CPDA ($P < .05$). There was no difference between sodium concentration in ACD at either time point (Figure 3A). Potassium concentration was significantly higher in bags with ACD compared to CPDA ($P < .01$ for both timepoints) but there was no difference between the time points with each anticoagulant (Figure 3B).

Oxygen concentration was significantly higher in bags with ACD compared to CPDA at collection ($P < .05$) but there was no significant difference between concentrations in the 2 anticoagulants after one hour. Oxygen concentration increased significantly after one hour in bags with ACD ($P < .05$) but was not significantly different after one hour in bags with CPDA (Figure 4A). Carbon dioxide concentration was significantly higher in ACD bags compared to CPDA ($t = 0$, $P < .0001$; $t = 1$, $P < .001$) but there was no difference between concentrations after one hour in each anticoagulant (Figure 4B).

Discussion

This study aimed to identify whether ACD or CPDA provides the better environment for maintaining equine PLT viability in PRP following whole blood collection.

Specifically, maximum PLT viability requires a highly standardized venipuncture technique to minimize spontaneous PLT activation, and an adequate anticoagulant and preservative to prevent coagulation
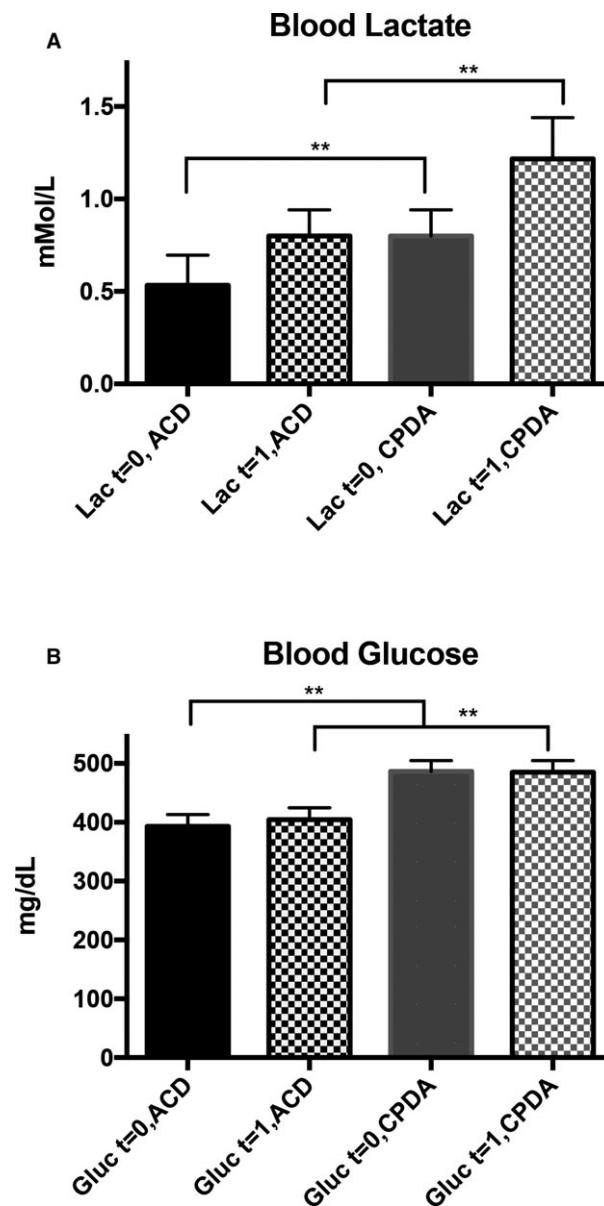
Figure 2. Means of lactate concentration (**A**) and blood glucose concentration (**B**) measured in healthy equine blood preserved/stored in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose-adenosine (CPDA) for one hour ($t = 1$). (**A**) Lactate concentration (mM/L) was significantly higher in CPDA compared to ACD at the time of collection ($t = 0$) and remained significantly higher after one hour. Lactate concentration increased significantly after one hour in CPDA and ACD. (**B**) Blood glucose concentration (mg/dL) was significantly higher in CPDA compared to ACD at the time of collection. Blood glucose concentration also remained significantly higher after one hour. Blood glucose concentration increased significantly after one hour in ACD but not in CPDA. *$P < .05$; **$P < .01$.

and grant optimal PLT function. The collection method described in this study was consistent for every sample, avoiding shear damage to the PLTs by using a low-gauge needle for collection followed by gentle
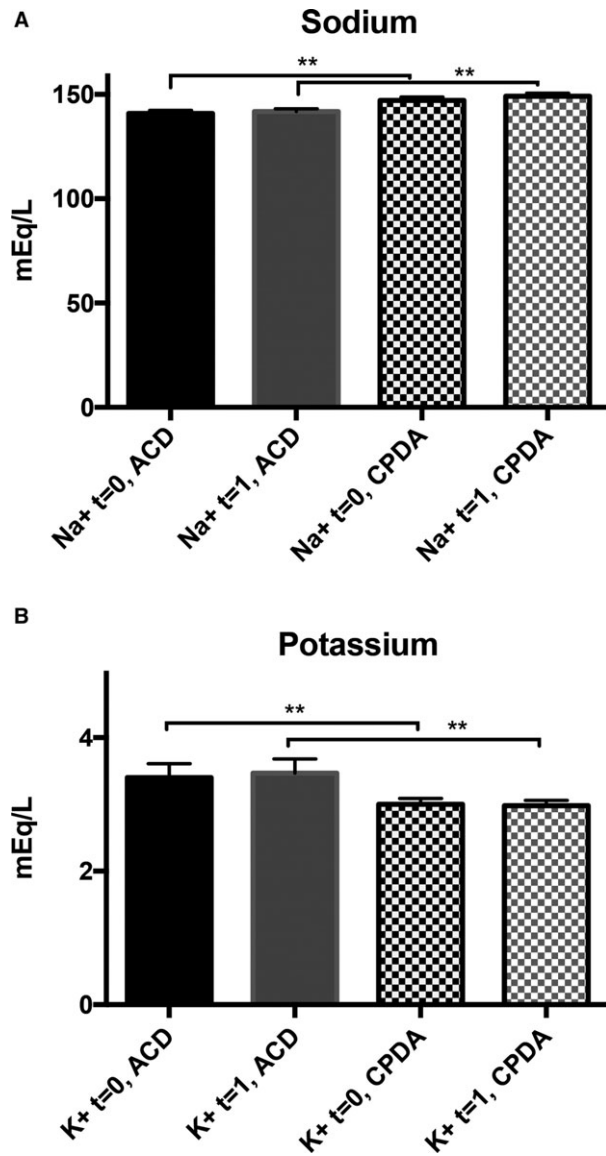
Figure 3. Means of sodium concentration (**A**) and potassium concentration (**B**) measured in healthy equine blood preserved/stored in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose-adenosine (CPDA) for one hour ($t = 1$). (**A**) Sodium concentration (mEq/L) was significantly higher in CPDA compared to ACD at the time of blood collection ($t = 0$) and after one hour, but not in ACD. (**B**): Potassium concentration (mEq/L) was significantly higher in ACD compared to CPDA at the time of blood collection, and remained significantly higher in ACD after one hour. There was no significant change in potassium concentration in ACD or CPDA after one hour. *$P < .05$; **$P < .01$.

Figure 4. Means of oxygen concentration (**A**) and carbon dioxide concentration (**B**) measured in healthy equine blood preserved/stored in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose-adenosine (CPDA) for one hour ($t = 1$). (**A**) Oxygen concentration (mmHg) was significantly higher in ACD compared to CPDA at the time of blood collection ($t = 0$). After one hour, oxygen concentration in CPDA and ACD were not significantly different. No significant difference was noted in oxygen concentration in CPDA at the time of collection compared to after one hour. However, oxygen concentration did increase significantly after one hour in ACD. (**B**) Carbon dioxide concentration (mmHg) was significantly higher in ACD compared to CPDA at the time of blood collection. Carbon dioxide concentration remained higher in ACD after one hour. There was no significant change in carbon dioxide concentration between blood collection and after one hour in ACD or CPDA. *$P < .05$; **$P < .01$.

handling. The PLT count variability that we determined may have been due to the inherent tendency of PLTs to aggregate and disaggregate with gentle manipulation and time, in spite of all the care applied.

Sodium citrate is considered superior to ACD for preserving structural and physiologic properties
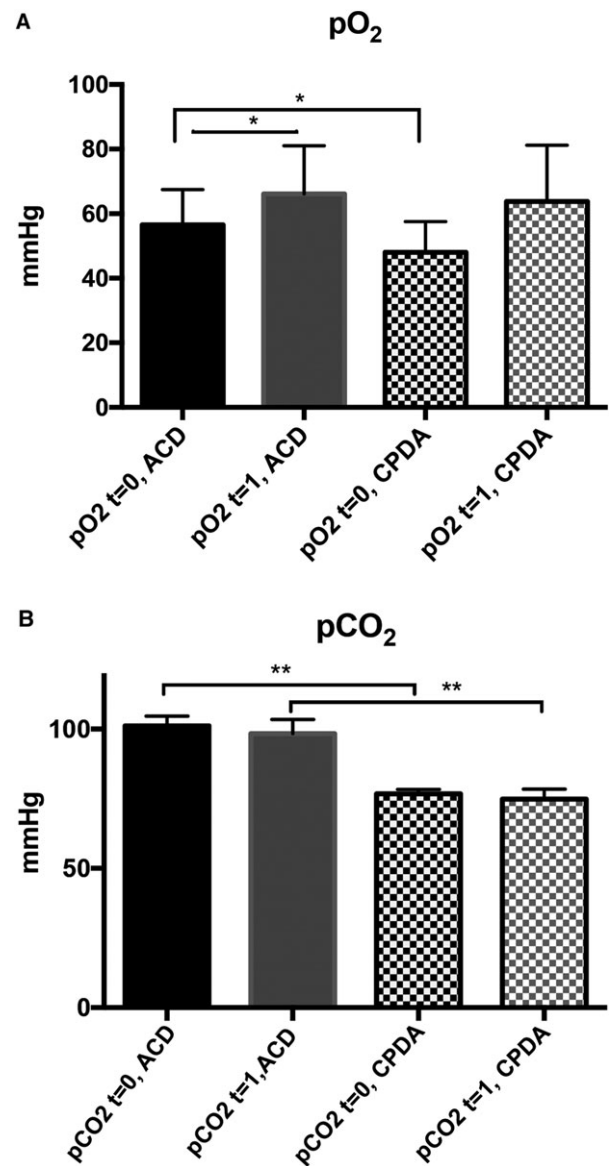
of human PLTs after 2 or more hours postblood collection[13]; however, little is known about CPDA's ability to preserve equine PLT function in whole

blood. Based on the significantly greater amplitude in PLT aggregometry in CPDA, we assume optimized PLT function and viability following collection of blood in CPDA compared to ACD. In vitro testing of PLT metabolic status has traditionally relied upon measurement of concentrations of glucose, lactate, pH, oxygen, carbon dioxide, and bicarbonate. The mean lactate, glucose, and bicarbonate concentrations were consistently higher in CPDA compared to ACD. The bicarbonate concentrations significantly decreased after one hour in both anticoagulants suggesting some storage-associated PLT damage in both anticoagulants, and higher lactate concentrations and lower bicarbonate and glucose concentrations are suggestive of PLT storage injury.[10] Glucose is required for PLT metabolism, but can be associated with increased lactate production and decreased pH triggering PLT storage lesion. While the pH was consistently lower in ACD compared to CPDA but did not change in either anticoagulant after one hour it appears that the pH was not associated with anaerobic glycolysis and lactate production. Likewise, despite significantly increased lactate concentrations, the pH did not decrease after one hour in either anticoagulant. Human PLTs exposed to a pH < 6.3 exhibit morphologic changes and have diminished in vivo survival upon transfusion.[14] Hence, the more neutral pH of CPDA may have contributed to improved PLT viability. Although the samples collected in both anticoagulants maintained a pH < 7.4, neither decreased to pH < 6.3. The higher lactate concentrations in CPDA compared to ACD are presumably due to higher metabolic activity[6] and may be associated with the lower oxygen concentrations in CPDA necessitating anaerobic metabolism. The higher (more neutral) pH in CPDA may have contributed to the increased metabolic activity and therefore higher lactate concentrations, as glycolysis is dependent on pH.[15] Indeed, lower pO_2 in initial CPDA samples may reflect the initial higher cellular metabolic activity in CPDA-preserved blood. As the temperature of the samples decreased from body temperature (37°C) to the one-hour storage temperature (22°C), metabolic activity may have lessened, and oxygen exchange across the gas permeable bags might have occurred resulting in an increase in pO_2.

Platelet aggregometry results from one horse were excluded, as the PLTs did not respond to activation by the physiologic agonist thrombin, in either anticoagulant. It is suspected that this may be due to historic medication with phenylbutazone or aspirin, or the presence of microclots in the samples prior to evaluation. Although every effort was made to select horses with no recent history of nonsteroidal anti-inflammatory medication for the study, a definitive assessment was not possible.

The purpose of this study was to evaluate PLTs stored in whole blood for one hour, as our hospital does not maintain equine PRP products for transfusion. For the aggregometry assay, we chose to use thrombin, the most potent physiologic agonist, although there may well have been altered responses to other agonists, including ADP and collagen. Small changes in lactate levels have been shown to affect PLT responses to agonists, in particular ADP.[16] As the aggregometry studies were conducted simultaneously with chemistry studies and without prior knowledge of lactate levels, thrombin was chosen as the aggregometry agonist.

A major limitation of this study is the small sample size, which makes results difficult to interpret. Furthermore, the PLT metabolic status cannot be reliably assessed in whole blood samples where other cells than PLTs are utilizing substrates and producing metabolic products. Another limitation is that calcium concentrations could not be measured due to the anticoagulants used and their effect on the blood gas analyzer reagents required for calcium measurements. Measurement of calcium may have aided result interpretation, as unphysiologic low calcium ion concentrations would inhibit PLT activation, and related release of intracellular contents and irreversible aggregation. Therefore, maintenance of PLT viability may also depend on calcium concentrations.[14]

Although interpretation of the metabolic status of the PLTs in this study is compounded by the presence of RBCs and WBCs in the whole blood samples, results of the aggregometry indicate that CPDA preserves PLT viability better than ACD, and should therefore be used for whole blood collection of PLTs determined for treatment of horses needing viable PLT. The study was adapted to the current transfusion practices in our teaching hospital, with a maximal lag time between collection and transfusion of about one hour. Further studies are indicated to assess PLT viability in purified PLTs stored in different anticoagulants over longer time periods to further substantiate these results.

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References

1. Castelijns G, Crawford A, Schaffer J, Ortolano GA, Beauregard T, Smith RKW. Evaluation of filter-prepared platelet concentrate for the treatment of suspensory branch injuries in horses. *Vet Comp Orthop Traumatol*. 2011;24:363–369.

2. Carmona JU, López C, Sandoval JA. Review of the currently available systems to obtain platelet related products to treat equine musculoskeletal injuries. *Recent Pat Regen Med*. 2013;3:148–159.

3. López C, Carmona JU. Platelet-rich plasma as an adjunctive therapy for the management of a severe chronic distal limb wound in a foal. *J Equine Vet Sci*. 2014;34:1128–1133.

4. Lacopetti I, Perazzi A, Ferrari V, Busetto R. Application of platelet-rich gel to enhance wound healing in the horse: a case report. *J Equine Vet Sci*. 2012;32:123–128.

5. Durham AE. Blood and plasma transfusion in the horse. *Equine Vet Educ*. 1996;8:8–12.

6. Mudge MC, MacDonald MH, Owens SD, Tablin F. Comparison of 4 blood storage methods in a protocol for equine pre-operative autologous donation. *Vet Surg*. 2004;33:475–486.

7. Giraldo CE, Álvarez ME, Carmona JU. Effects of sodium citrate and acid citrate dextrose solutions on cell counts and growth factor release from equine pure-platelet rich plasma and pure-platelet rich gel. *BMC Vet Res*. 2015;11:60.

8. Beutler E. Erythrocyte metabolism and its relation to the liquid preservation of blood. In: Petz LD, Swisher SN, eds. *Clinical Practice of Transfusion Medicine*. New York, NY: Churchill Livingstone; 1989:271–296.

9. Bucheler J, Cotter SM. Storage of feline and canine whole blood in CPDA-1 and determination of post-transfusion viability. *J Vet Intern Med*. 1994;8:172.

10. Hoareau GL, Jandrey KE, Burges J, Bremer D, Tablin F. Comparison of the platelet-rich plasma and buffy coat protocols for preparation of canine platelet concentrates. *Vet Clin Pathol*. 2014;43:513–518.

11. Snyder EL, Hezzey A, Katz AJ, Bock J. Occurrence of the release reaction during preparation and storage of platelet concentrates. *Vox Sang*. 1981;41:172–177.

12. Filip DJ, Aster RH. Relative hemostatic effectiveness of human platelets stored at 4 degrees and 22 degrees C. *J Lab Clin Med*. 1978;91:618–624.

13. Macey M, Azam U, McCarthy D, et al. Evaluation of the anticoagulants EDTA and citrate, theophylline, adenosine, and dipyridamole (CTAD) for assessing platelet activation on the ADVIA 120 hematology system. *Clin Chem*. 2002;48:891–899.

14. Perrotta PL, Snyder EL. Platelet storage and transfusion. In: Michelson AD, ed. *Platelets*. Philadelphia, PA: Elsevier Inc.; 2007:1265–1295.

15. Högman CF. Liquid storage of human erythrocytes. In: Harris JR, ed. *Blood Separation and Plasma Fractionation*. New York, NY: Wiley-Liss; 1991:63–97.

16. Kofoed KF, Naesh O, Hindberg I, Winther K. Attenuated platelet aggregation in the presence of high concentrations of lactate. *Thromb Res*. 1991;63:549–552.