Effects of anticoagulant on pH, ionized calcium concentration, and agonist-induced platelet aggregation in canine platelet-rich plasma

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Objective—To compare effects of 3.8% sodium citrate and anticoagulant citrate dextrose solution National Institutes of Health formula A (ACD-A) on pH, extracellular ionized calcium (iCa) concentration, and platelet aggregation in canine platelet-rich plasma (PRP).

Sample Population—Samples from 12 dogs.

Procedures—Blood samples were collected into 3.8% sodium citrate (dilution, 1:9) and ACD-A (dilution, 1:5). Platelet function, pH, and iCa concentration were evaluated in PRP. Platelet agonists were ADP, γ-thrombin, and convulxin; final concentrations of each were 20μM, 100nM, and 20nM, respectively. Washed platelets were used to evaluate effects of varying the pH and iCa concentration.

Results—Mean pH and iCa concentration were significantly greater in 3.8% sodium citrate PRP than ACD-A PRP. Platelet aggregation induced by ADP and γ-thrombin was markedly diminished in ACD-A PRP, compared with results for 3.8% sodium citrate PRP. Anticoagulant had no effect on amplitude of convulxin-induced platelet aggregation. In washed platelet suspensions (pH, 7.4), there were no differences in amplitude of platelet aggregation induced by convulxin or γ-thrombin at various iCa concentrations. Varying the pH had no effect on amplitude of aggregation induced by convulxin or γ-thrombin, but the aggregation rate increased with increasing pH for both agonists.

Conclusions and Clinical Relevance—Aggregation of canine platelets induced by ADP and γ-thrombin was negligible in ACD-A PRP, which suggested an increase in extraplatelet hydrogen ion concentration inhibits signaling triggered by these agonists but not by convulxin. Choice of anticoagulant may influence results of in vitro evaluation of platelet function, which can lead to erroneous conclusions. (*Am J Vet Res* 2009;70:472–477).

Citrate, EDTA, and heparin are anticoagulants commonly used in clinical veterinary practice to prevent clotting of blood for various in vitro laboratory evaluations (plasma coagulation screens, CBCs, and acid-base analyses, respectively). A comparison of platelet variables in EDTA- and citrate-anticoagulated blood in dogs has revealed that platelet counts are lower and MPV higher in 3.2% citrate samples than in EDTA samples, presumably because of increased platelet activation and aggregation in citrate, with nonsphering of platelets in citrate also potentially causing an artifactual increase in MPV.[1] Citrate, which chelates positively charged calcium ions and thereby blocks calcium-dependent clotting factor reactions, is the anticoagulant typically used for collection of blood for transfusion as well as for in vitro evaluation of platelet function. Citrate is combined with preservative solutions (eg, citrate plus phosphate, dextrose, and adenine) to allow storage of blood components. The anticoagulant ACD-A is the choice for collection of platelets by apheresis from human[2] and canine[3] donors, whereas trisodium citrate (3.2% or 3.8%) is the anticoagulant most commonly used for diagnostic evaluations of platelets.[4] Trisodium citrate and ACD-A solutions differ markedly in pH, with ACD-A having a pH of 4.9 and 3.8% sodium citrate having a pH of 7.8. In addition, the citrate ion concentration in ACD-A is 15.6 mg/mL, whereas 3.8% sodium citrate contains

Abbreviations

ACD-A	Anticoagulant citrate dextrose solution National Institutes of Health formula A
GP	Glycoprotein
iCa	Ionized calcium
MPV	Mean platelet volume
PGE_1	Prostaglandin E_1
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma

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24.4 mg of citrate ion/mL. It has been reported[5–11] in several species that alterations in the pH and extracellular iCa concentration of PRP can affect platelet aggregation in vitro, with aggregation typically impaired at acidic pH and lower extracellular iCa concentrations.

In vitro analysis of platelet aggregation may be part of the evaluation of a patient with suspected thrombopathia or a quality-control procedure for canine platelet transfusion products, such as apheresis platelet concentrates and cryopreserved platelets. The purpose of the study reported here was to compare the effects of 3.8% sodium citrate and ACD-A on pH, extracellular iCa concentration, and platelet aggregation in canine PRP.

Materials and Methods

Animals—Twelve healthy adult mixed-breed dogs (7 sexually intact females that were not pregnant or in estrus at the time of blood collection and 5 sexually intact males) were used in the study. Dogs were selected for use in the study on the basis of results of a complete physical examination and a CBC. The dogs were not receiving any medications. The dogs were housed in a research colony at the University of Pennsylvania School of Veterinary Medicine. Food was withheld from all dogs for 12 hours prior to collection of blood samples, but dogs had ad libitum access to water. The study was performed in compliance with institutional guidelines for research on animals.

Collection of blood samples and preparation of platelets—Blood samples were collected with minimal trauma from a jugular vein via an 18-gauge apheresis needle[a] into syringes containing 3.8% sodium citrate (1 mL of citrate:9 mL of blood) or ACD-A[b] (1 mL of ACD-A: 5 mL of blood); these differing anticoagulant-to-blood ratios result in an almost equal concentration of citrate ion in the anticoagulated whole blood (2.44 and 2.6 mg of citrate ion/mL of whole blood for the anticoagulants 3.8% sodium citrate and ACD-A, respectively). Platelet count and MPV were measured in anticoagulated whole blood by use of an automated hematology analyzer.[c] The PRP was prepared by centrifugation of whole blood (650 × *g* at 22°C; 2 times for 3 min/centrifugation). The resultant PRP was pooled, and platelet number was determined by use of an automated hematology analyzer[d] and adjusted to a concentration of 300,000 platelets/µL by the addition of autologous PPP obtained by centrifugation of blood samples at 2,200 × *g* for 15 minutes. Platelet-free plasma was subsequently prepared by centrifugation of PPP at 16,000 × *g* for 20 minutes. The PRP was allowed to sit undisturbed at 22°C for 30 minutes prior to use.

For preparation of washed platelets, PGE_1 (final concentration, 1µM) was added to PRP, which was then centrifuged at 16,000 × *g* at 22°C for 20 seconds. The resultant platelet pellets were then washed twice in platelet wash buffer (113mM NaCl, 4.3mM K_2HPO_4, 4.2mM Na_2HPO_4, 24.4mM NaH_2PO_4, 5.5mM glucose, and 1µM PGE_1 [pH, 6.3]).[12] After the second wash, platelets were resuspended at a final concentration of 300,000 platelets/µL in resuspension buffer (137mM NaCl, 4 mM KCl, 0.5mM $MgCl_2$, 0.5mM Na_2HPO_4, 0.1% glucose, 0.1% bovine serum albumin, and 10mM HEPES)[12] at various pH values (7.0, 7.2, and 7.4) and extracellular iCa concentrations (0.12, 0.2, and 0.5 mmol/L), with the latter achieved by use of a stock solution of 1.5M $CaCl_2$. The pH and iCa concentration of the PRP and platelet resuspension buffers were measured by use of an automated analyzer with an ion-selective electrode.[e]

Evaluation of platelet aggregation—Platelet aggregation was measured by use of a turbidimetric technique in a dual-channel aggregometer[f] coupled to a computer interface[g] for display of aggregation curves and calculation of amplitude (maximum increase in light transmittance, expressed as a percentage) and slope (rate of aggregation, expressed as the percentage increase in light transmittance per minute). The PPP or platelet-resuspension buffer was used to determine a light transmittance of 100%. Then, 500 µL of PRP or washed platelets (490 µL of washed platelets, 5 µL of platelet-free plasma, and 5 µL [0.5 units] of hirudin[h]) was pipetted into a 1-mL siliconized glass cuvette and warmed to 37°C for 1 minute with stirring (1,000 revolutions/min). We determined that this concentration of hirudin completely blocked generation of thrombin after addition of platelet-free plasma to washed platelets, as indicated by a complete flatline response (ie, no platelet aggregation or change in platelet shape during a 10-minute monitoring period). In addition, this concentration of hirudin did not cause inhibition of agonist-induced aggregation. Agonists were then added to the cuvettes (ADP[i] at a final concentration of 20µM; γ-thrombin[j] at a final concentration of 100nM, convulxin[k] at a final concentration of 20nM, and U46619[l] at final concentrations of 1 and 10µM). In addition, to determine whether a subthreshold concentration of convulxin (final concentration, 1.2nM) could potentiate aggregation induced by γ-thrombin (final concentration, 50nM) in ACD-A PRP, both agonists were added concurrently to PRP obtained from 2 dogs. The extent of platelet aggregation was measured at plateau, which was typically 4 to 5 minutes after addition of both agonists.

Statistical analysis—For pairwise comparisons of PRP anticoagulated with ACD-A and 3.8% sodium citrate, a paired *t* test was performed. To determine effects of varying the pH and extracellular iCa concentrations on aggregation of washed platelets, a repeated-measures ANOVA was used. Data were reported as mean ± SD, unless otherwise indicated. All analyses were performed by use of statistical software.[m] A value of $P < 0.05$ was considered significant.

Results

Hct and platelet number—The Hct for healthy dogs in the study ranged from 40% to 46% (reference range, 40% to 60%). Platelet counts for the dogs ranged from 217,000 to 314,000 platelets/µL (reference range, 177,000 to 398,000 platelets/µL).

Effect of anticoagulant on platelet count and MPV in whole blood—The mean ± SD platelet count in whole blood anticoagulated with ACD-A (256,000 ± 26,000 platelets/µL) did not differ significantly from that of whole blood anticoagulated with 3.8% sodium citrate (262,000 ± 34,000 platelets/µL). Similarly, there

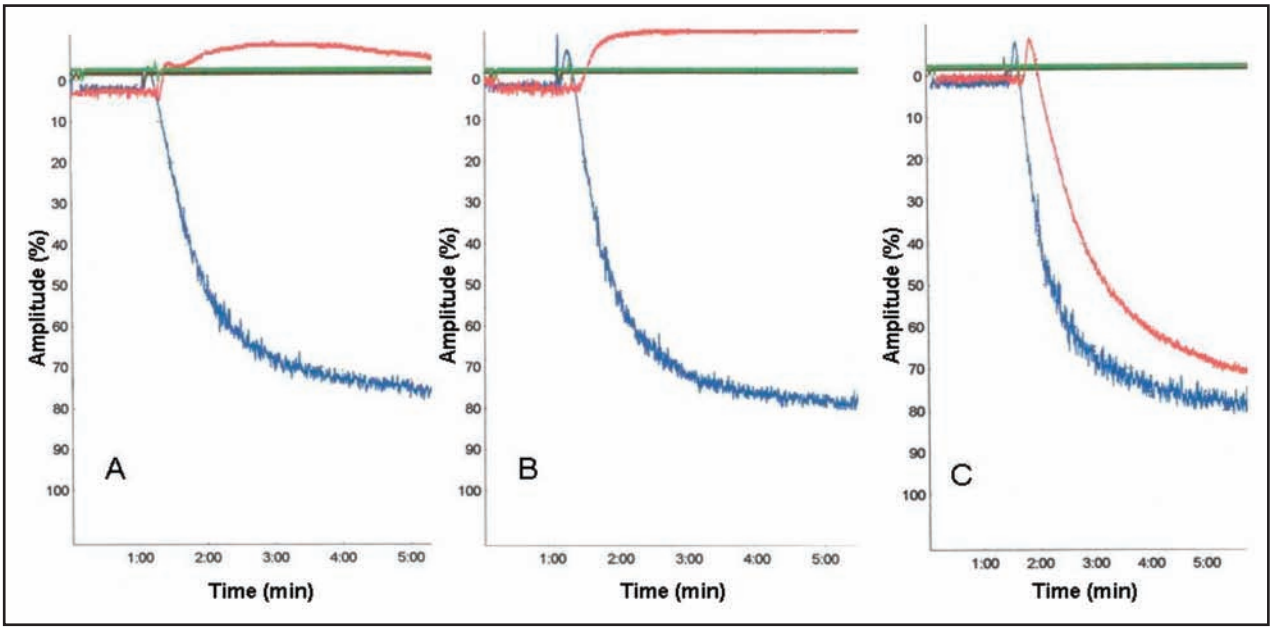

Figure 1—Aggregation responses (amplitude, which was expressed as a percentage increase in light transmittance) induced by use of 20μM ADP (A), 100nM γ-thrombin (B), and 20nM convulxin (C) in canine PRP anticoagulated with 3.8% sodium citrate (blue line) and ACD-A (red line). Tracings are for samples obtained from a representative dog.

was no significant difference in MPV between whole blood samples collected into ACD-A (7.4 ± 1.6 fL) or 3.8% sodium citrate (6.9 ± 0.9 fL).

Effect of anticoagulant on pH and extracellular iCa concentration in PRP—The ACD-A was more acidic (mean ± SD pH, 7.05 ± 0.06) than 3.8% sodium citrate PRP was more alkaline (pH, 7.56 ± 0.04); these values differed significantly ($P < 0.001$). Mean extracellular iCa concentration was significantly ($P < 0.001$) higher in 3.8% sodium citrate PRP (0.22 ± 0.02 mmol/L) than in ACD-A PRP (0.19 ± 0.01 mmol/L).

Effect of anticoagulant on platelet aggregation in PRP—Platelet aggregation induced by ADP was markedly diminished in ACD-A PRP, compared with that for 3.8% sodium citrate PRP; in addition, platelets from 9 of 12 dogs had only a change in shape in ACD-A PRP **(Figures 1 and 2)**. Platelets collected in ACD-A from all 12 dogs responded to γ-thrombin, with only a change in platelet shape. This was in contrast to platelets in 3.8% sodium citrate, which had a mean increase in light transmittance of 66%. Anticoagulant did not have an effect on platelet aggregation induced by convulxin; however, the slope (rate of aggregation) was significantly ($P = 0.03$) less in ACD-A PRP (mean ± SE, 56 ± 6%) than in 3.8% sodium citrate PRP (77 ± 10%). The combination of a subthreshold final concentration of 1.2nM convulxin and 50nM γ-thrombin, each of which separately resulted in only a change in platelet shape in the ACD-A PRP from 2 dogs, resulted in amplitudes of 65% and 75%, which indicated a potentiating effect of convulxin on platelet aggregation induced by γ-thrombin in the face of an acidic plasma pH. Use of U46619 (1 and 10μM) induced a change in platelet shape but no aggregation in 3.8% sodium citrate PRP from all dogs, whereas there was no detectable change in platelet shape in ACD-A PRP and weak (< 10%) microaggregate formation that plateaued within 2 minutes.

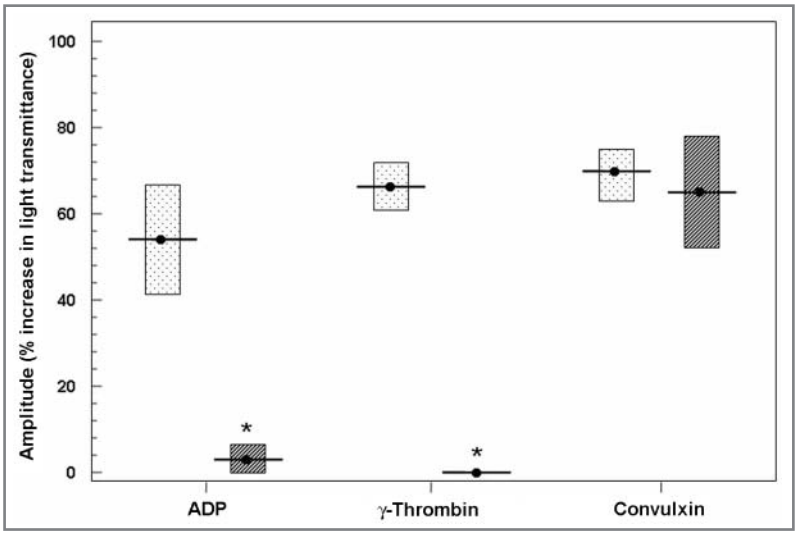

Figure 2—Mean (horizontal line) ± 2 SE (limits of the boxes) amplitude (expressed as a percentage of increase in light transmittance) of agonist-induced platelet aggregation (20μM ADP, 100nM γ-thrombin, and 20nM convulxin) in samples of canine PRP anticoagulated with 3.8% sodium citrate (stippled boxes) and ACD-A (diagonal-striped boxes). Results represent analysis of samples obtained from 12 dogs. *Within an agonist, mean values differ significantly ($P < 0.001$) between anticoagulants.

Effects of extracellular iCa concentration and pH on aggregation of washed platelets—In washed platelet suspensions at a pH of 7.4, no differences were de-

Table 1—Mean ± SE agonist-induced aggregation of washed canine platelets for various extracellular iCa concentrations.

		Extracellular iCa (mmol/L)		
Agonist	**Variable**	**0.12**	**0.20**	**0.50**
γ-Thrombin (50nM)	Amplitude (%)	63 ± 1	62 ± 1	64 ± 1
	Slope (%/min)	69 ± 3	67 ± 3	65 ± 3
γ-Thrombin (100nM)	Amplitude (%)	71 ± 2	73 ± 2	75 ± 2
	Slope (%/min)	101 ± 4	115 ± 4*	117 ± 4†
Convulxin (20nM)	Amplitude (%)	58 ± 2	56 ± 2	60 ± 2
	Slope (%/min)	90 ± 4	91 ± 4	94 ± 4

Results represent analysis of samples obtained from 12 dogs. All aggregations were conducted at a pH of 7.4.

*Within a row, value differs significantly ($P = 0.02$) from the value for 0.12 mmol/L. †Within a row, value differs significantly ($P = 0.008$) from the value for 0.12 mmol/L.

Table 2—Mean ± SE agonist-induced aggregation of washed canine platelets for various pH values.

		pH		
Agonist	**Variable**	**7.0**	**7.2**	**7.4**
γ-Thrombin (50nM)	Amplitude (%)	40 ± 7	47 ± 7	64 ± 7*
	Slope (%/min)	19 ± 6	31 ± 6	65 ± 6†
γ-Thrombin (100nM)	Amplitude (%)	64 ± 4	72 ± 4	75 ± 4
	Slope (%/min)	64 ± 7	78 ± 7	117 ± 7†
Convulxin (20nM)	Amplitude (%)	54 ± 4	54 ± 4	60 ± 4
	Slope (%/min)	75 ± 5	78 ± 5	94 ± 5‡

Results represent analysis of samples obtained from 12 dogs. All aggregations were conducted at an extracellular iCa concentration of 0.50 mmol/L.

*Within a row, value differs significantly ($P = 0.03$) from the value for a pH of 7.0. †Within a row, value differs significantly ($P < 0.001$) from the value for a pH of 7.0 and a pH of 7.2. ‡Within a row, value differs significantly ($P < 0.05$) from the value for a pH of 7.0 and a pH of 7.2.

tected in the amplitude of platelet aggregation induced by convulxin or γ-thrombin at various iCa concentrations, although the slope increased with increasing iCa concentrations in response to γ-thrombin (**Table 1**). Variation in pH had no effect on the amplitude of aggregation induced by convulxin or 100nM γ-thrombin (**Table 2**). However, use of a lower final concentration of γ-thrombin (50nM) resulted in an amplitude significantly less at a pH of 7.0 than at a pH of 7.4. The slope of aggregation increased with increasing pH with both agonists.

Discussion

Choice of anticoagulant (EDTA or 3.2% sodium citrate) can affect platelet count and MPV in canine whole blood.[1] However, different citrate formulations (3.8% sodium citrate and ACD-A) in the study reported here yielded similar platelet counts and MPV. However, the choice of anticoagulant (3.8% sodium citrate or ACD-A) can greatly influence in vitro platelet aggregation responses in canine PRP. The platelet agonists used in our study were selected to evaluate the effects of pH and extracellular iCa concentration on various platelet activation pathways. With the exception of collagen, most platelet agonists trigger platelet activation by binding to G-protein–coupled receptors on the platelet surface.[13] Collagen binds directly or indirectly to at least 4 molecules or molecular complexes on the platelet surface (ie, GP Ib/IX, $\alpha_2\beta_1$, $\alpha_{IIb}\beta_3$, GP IV, and GP VI).[13] In the authors' experience, there is a variable response of canine platelets to commercial collagen preparations, which typically consist of type I collagen from equine tendons. Also, in contrast to responses in human platelets, 6- to 12-fold higher doses of collagen are required to elicit similar aggregation and secretion responses in canine PRP.[14] Therefore, convulxin, a potent platelet aggregating protein isolated from *Crotalus durissus terrificus* venom that binds to and activates the platelet GP VI collagen receptor,[15] was used in place of collagen. Experiments with human platelets have revealed that convulxin can induce changes in platelet shape through a calcium-independent pathway mediated by Rho kinase.[16] Human γ-thrombin, a nonclotting derivative from thrombin resulting from controlled incubation with trypsin-sepharose, retains its platelet-activating capacity without resulting in fibrin clot formation.[17] When studied in a citrated PRP system, the stable thromboxane A_2 analogue, U46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$), failed to induce irreversible aggregation and secretion, with most canine platelets being thromboxane insensitive because of impaired activation of phospholipase C secondary to a receptor-linked G-protein dysfunction.[18]

The most striking difference between 3.8% sodium citrate and ACD-A PRPs in the study reported here was for pH, although there was a significant but relatively small difference for extracellular iCa concentration. In the ACD-A PRP with a mean pH of 7.05, there was almost complete inhibition of platelet aggregation in response to 20μM ADP and 100nM γ-thrombin. In contrast, in the 3.8% sodium citrate PRP with a mean pH of 7.56, there was an increase in light transmittance of approximately 60% in response to these 2 agonists. Interestingly, the anticoagulant did not have an effect on the extent of platelet aggregation induced by convulxin, with an increase in light transmittance of > 60% in both ACD-A PRP and 3.8% sodium citrate PRP. However, the slope (ie, rate) of convulxin-induced platelet aggregation was increased in 3.8% sodium citrate PRP. Analysis of results of this study indicated that increased extraplatelet hydrogen ion concentrations inhibit signaling triggered by ADP and γ-thrombin but not signaling triggered by convulxin. As reported in another study,[18] U46619 (1 and 10μM) results in only a change in platelet shape in 3.8% sodium citrate PRP, which indicates that U46619 binds to the thromboxane A_2 receptor on the surface of canine platelets but fails to signal aggregation. Interestingly, U46619 failed to elicit a discernable change in platelet shape in ACD-A PRP, which suggested that the U46619-induced response in shape change is a pH-sensitive event. However, the higher hydrogen ion concentration in ACD-A PRP favored microaggregate formation in response to U46619, which suggested that in an environment with a lower pH (7.0 vs 7.5), this thromboxane mimic can activate signaling pathways in canine platelets that lead to weak activation of fibrinogen receptors. Future evaluation of canine platelet G-proteins could provide information on the effects of pH and iCa concentrations on platelet signaling.

To separately evaluate the effects of pH and extracellular iCa concentration on aggregation in canine plate-

lets, washed platelet suspensions were prepared. Use of ADP did not provide consistent aggregation responses with the washed platelet suspensions, potentially because of ADP being released from the platelets during washing and a resulting platelet refractoriness attributable to selective desensitization of the $P2Y_1$ ADP receptor.[19] Apyrase was added to PRP and the first platelet wash to scavenge ADP, but there was no improvement in response. Therefore, only data for washed platelets with convulxin and γ-thrombin as agonists were reported. Preliminary experiments with washed canine platelets at various extracellular iCa concentrations did not reveal significant differences between extent of aggregation at concentrations of 0.5, 1, and 2 mmol/L; therefore, an iCa concentration of 0.5 mmol/L was selected as the maximum concentration for this study. Although increasing the iCa concentration resulted in an increase in the slope of aggregation of washed platelets in response to 100nM γ-thrombin, the effect of pH on rate of aggregation was more marked, with the slope of platelet aggregation induced by 50nM γ-thrombin being > 3-fold higher at a pH of 7.4, compared with the slope at a pH of 7.0. Overall, the pH and iCa concentration effects on platelet aggregation detected in the washed platelet suspensions were less dramatic than those detected in PRP. The combination of low pH and low iCa concentration (for example, a pH of 7.0 and iCa concentration of 0.2 mmol/L) in ACD-A PRP was not evaluated in washed platelet suspensions; it is possible that the combination of these 2 factors may have resulted in a more marked impairment of platelet aggregation in the washed suspensions. In addition, the effect of low pH on plasma proteins in the PRP that could have influenced platelet aggregation was not a consideration in the washed platelet preparations.

Studies have confirmed the deleterious effect of low pH on in vitro aggregation of platelets obtained from rabbits[9] and humans.[5–7] The phenomenon of acidosis-induced inhibition of human platelet aggregation has been attributed to inhibition of store-operated calcium influx (ie, the calcium entry pathway that is activated by depletion of intracellular calcium stores) by acidosis (pH, 6.9).[6] A plasma pH of 7.3 to 7.6 appears optimal for evaluation of in vitro aggregation with human platelets[5,8] because decreased aggregation in response to ADP and thrombin has been detected at pH > 7.6.[5] Analysis of results of the study reported here indicated that aggregation induced by ADP and γ-thrombin, but not changes in platelet shape, in canine PRP is similarly a pH-dependent event, with the ACD-A PRP at a pH of 7.05 having negligible aggregation, whereas normal aggregation responses were detected in 3.8% sodium citrate PRP at a pH of 7.56.

In addition to pH, the extracellular concentration of divalent cations has an effect on in vitro aggregation of platelets from various species. Addition of EDTA to a suspension of washed human platelets or omission of calcium and magnesium from the fluid used to suspend the platelets prevented aggregation by ADP, although there was no change in shape.[10] The presence of calcium or magnesium in the suspension buffer supports ADP-induced aggregation of human platelets[10]; however, the presence of magnesium, either alone or in combination with calcium, is necessary for both primary and secondary waves of platelet aggregation.[20] Species differences exist because ADP can induce a change in shape but no aggregation of washed rabbit platelets when resuspended in a medium containing magnesium but without added calcium.[10] The platelet resuspension buffer used in the study reported here for the washed canine platelets contained 0.5mM $MgCl_2$; effects of the absence of this divalent cation or variations in its concentration on canine platelet aggregation were not evaluated in this study.

The influence of extracellular iCa concentration in citrated PRP has carried over to the preclinical and clinical setting for humans. For example, there is substantial evidence that platelets in citrated PRP from female donors have greater aggregation in response to epinephrine, collagen, and ADP than do platelets from male donors.[21] The difference in aggregation of human platelets associated with sex of the donor has been attributed to the greater extracellular iCa concentration in the citrated PRP of females that resulted when a fixed volume of citrate was used to prevent coagulation of blood; the lower Hct of female donors yielded a greater dilution of citrate in the plasma, compared with the yield for male donors.[21] More importantly, the effect of citrate on reducing plasma iCa concentrations can influence ex vivo platelet inhibition by GP IIb/IIIa receptor antagonists in humans and dogs, thereby potentially providing a false indication of the inhibitory potency of such drugs.[22–24] Antagonism of the platelet fibrinogen receptor GP IIb/IIIa by various pharmacologic agents has been helpful in reducing thrombotic complications of angioplasty and acute coronary syndromes in human patients. Preclinical evaluation of GP IIb/IIIa receptor antagonists relies on ex vivo platelet inhibition assays. Several studies[22–24] have confirmed that citrate anticoagulation removes iCa from GP IIb/IIIa, thereby enhancing the inhibitory activity of some GP IIb/IIIa receptor antagonists (primarily class II, including eptifibatide and orbofiban) and potentially overestimating the antithrombotic efficacy of these drugs.

Although anticoagulant-induced changes in pH and iCa concentration can have a substantial effect on in vitro evaluation of platelet aggregation, the collection and storage of platelets for transfusion in the anticoagulant-preservative solution ACD-A is not problematic. In fact, in addition to the iCa-lowering effect of citrate that allows collection of plasma-containing products without fibrin clot formation, the low pH of ACD-A is helpful in preventing premature platelet activation during apheresis collection and storage.[25] Furthermore, the pH of human platelet concentrates can decrease during storage because of anaerobic glycolysis, which results in an increase in lactic acid production, with a substantial loss of in vitro function at pH ≤ 6.2.[26] Experimental manipulation of human platelet concentrates to create transient adverse metabolic conditions (pH < 6.0 for 1 to 2 hours) results in severe in vitro functional derangements, including loss of resting morphology, complete abrogation of platelet aggregation, and decreased release of α-granules.[26] However, rescue from the acidotic state to an optimal pH by addition of autologous plasma improves morphology scores, restores platelet ag-

gregation and secretion, and results in posttransfusion platelet survival times comparable to those of control platelets, which indicates that the effects of transient severe metabolic stress on platelets are reversible.[26] In addition, there is ample evidence that stored platelets have the ability to rapidly reverse some aspects of the in vitro platelet storage lesions following transfusion. Administration of autologous apheresis human platelet concentrates with a mean pH of 6.97 after storage for 8 days at 22°C resulted in a mean posttransfusion survival time of 5.5 days, which is considered to be of acceptable poststorage quality when compared with the survival time of 8.4 days for fresh platelet concentrate (pH, 7.38).[27]

Canine PRP prepared by use of the anticoagulants ACD-A (dilution, 1:5) and 3.8% sodium citrate (dilution, 1:9) differed markedly in pH and, to a lesser extent, extracellular iCa concentration. The negligible aggregation responses to ADP and γ-thrombin in canine ACD-A PRP (pH, 7.05) suggested that increased concentrations of extraplatelet hydrogen ions inhibit signaling triggered by these platelet agonists but not by convulxin. The choice of anticoagulant may influence in vitro evaluation of platelet function, which can lead to erroneous quality-control conclusions regarding suitability for use of a platelet product for transfusion support treatment.

a. SysLoc safety A.V. fistula needle set, JMS Singapore PTE Ltd, Singapore, Singapore.
b. ACD-A, Baxter-Fenwal, Deerfield, Ill.
c. Cell-Dyn 3700, Abbott Diagnostics, Abbott Park, Ill.
d. Scil Vet ABC, Scil Animal Care Co, Gurnee, Ill.
e. Stat Profile Critical Care Xpress, Nova Biomedical Corp, Waltham, Mass.
f. Model 560Ca, Chrono-log Corp, Havertown, Pa.
g. AGGRO/LINK, Chrono-log Corp, Havertown, Pa.
h. Sigma-Aldrich, St Louis, Mo.
i. Chrono-log Corp, Havertown, Pa.
j. Human γ-thrombin, Enzyme Research Laboratories, South Bend, Ind.
k. Pentapharm, Basel, Switzerland.
l. Biomol International, Plymouth Meeting, Pa.
m. SAS, version 9.1, SAS Institute Inc, Cary, NC.

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