

Characteristics of canine platelet-rich plasma prepared with five commercially available systems

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OBJECTIVE

To characterize platelet-rich plasma (PRP) products obtained from canine blood by use of a variety of commercially available devices.

SAMPLE

Blood samples from 15 dogs between 18 months and 9 years of age with no concurrent disease, except for osteoarthritis in some dogs.

PROCEDURES

PRP products were produced from blood obtained from each of the 15 dogs by use of each of 5 commercially available PRP-concentrating systems. Complete blood counts were performed on each whole blood sample and PRP product. The degree of platelet, leukocyte, and erythrocyte concentration or reduction for PRP, compared with results for the whole blood sample, was quantified for each dog and summarized for each concentrating system.

RESULTS

The various PRP-concentrating systems differed substantially in the amount of blood processed, method of PRP preparation, amount of PRP produced, and platelet, leukocyte, and erythrocyte concentrations or reductions for PRP relative to results for whole blood.

CONCLUSIONS AND CLINICAL RELEVANCE

The characteristics of PRP products differed considerably. Investigators evaluating the efficacy of PRPs need to specify the characteristics of the product they are assessing. Clinicians should be aware of the data (or lack of data) supporting use of a particular PRP for a specific medical condition. (*Am J Vet Res* 2015;76:822–827)

Numerous cytokines and growth factors stored within platelet alpha granules are potentially effective at mitigating inflammation and initiating anabolic processes and tissue regeneration.¹ As a result, PRP products are commonly used in human medicine for intra-articular treatment of osteoarthritis; for intralesional administration in the treatment of ligament, tendon, and muscle injury; and to augment bone regeneration in dentistry applications.^{2–4} With promising results reported from several clinical studies, numerous companies have produced PRP-concentrating systems intended for use with human blood.⁵ Accordingly, multiple investigations^{5–7} have been performed in an attempt to identify differences between systems, characterize the PRP obtained with each system, and ascertain clinical ramifications for the use of different PRP preparations. Particular interest has been dedicated to quantifying the platelet concentration, leukocyte concentration, and concentrations of specific cytokines and growth factors within the PRP. These characteristics have been extensively evaluated because

although platelets can be a source of numerous anabolic cytokines, platelets and particularly leukocytes can also release proinflammatory cytokines that exacerbate or perpetuate inflammation and potentially impede tissue healing.⁶

Comparisons among human PRPs have revealed variability in platelet, leukocyte, and growth factor concentrations.^{5–7} These results are important because they underscore the fact that results from a clinical investigation conducted by use of one PRP system cannot be assumed to be representative of results that would be obtained by use of another PRP system.¹ Similarly, the ideal PRP product for most ailments remains unclear, and even if one product were superior for treatment of one condition, it may not be ideal for all applications. As a result, additional studies are necessary to clarify the advantages and disadvantages of each PRP in regard to its clinical use in humans. Platelet-rich plasma is widely used in humans, and PRP use and investigation in dogs have increased.^{8–14} In turn, numerous companies are marketing PRP-concentrating systems, many of which are manufactured specifically for producing PRP from human blood, for use with canine blood. However, relatively little has been done to characterize PRP obtained from canine blood

ABBREVIATIONS

ACD Acid citrate dextrose
PRP Platelet-rich plasma

by use of these systems. In 1 randomized controlled study,⁸ investigators found that a filtration-based system successfully concentrated canine platelets 3-fold over baseline concentrations, but it also significantly increased the concentration of leukocytes (1.8 times baseline concentrations). Investigators of another study¹⁵ reported that a centrifugation-based device successfully concentrated platelets (mean of 6-fold increase over the concentration in the baseline blood sample), but no data were provided on the concurrent concentration or reduction of leukocytes in the samples. Finally, another centrifugation-based system yielded a PRP product that had a significant reduction in leukocytes, but the platelets were not concentrated above the concentration in whole blood.¹⁶ This is noteworthy, given that the same system successfully concentrated human platelets to 2 times the concentration in whole blood.¹⁶ Analysis of these data indicates that one cannot assume that a system will produce the same PRP from canine blood as that obtained from human blood. Further, these data highlight that the PRPs obtained by use of various commercially available systems can potentially differ in their characteristics.

The fact that PRPs can differ substantially is pertinent in that some *in vivo* clinical and experimental studies in dogs yielded results that have been considered disappointing.¹⁷⁻¹⁹ For example, investigators of 1 study¹¹ compared bone healing with an experimentally created ulnar defect in which dogs were randomly allocated to receive filling of the defect with calcium phosphate granules supplemented with PRP gel or filling with only the calcium phosphate granules. There was no difference in osseous regeneration between these treatment groups. However, characteristics of the PRP used remain unclear because although the investigators cite other studies that involved use of the same PRP preparation protocol, no assessment of the PRP was made in that study¹¹ and therefore no data were provided as to the platelet, leukocyte, and erythrocyte concentrations. Accordingly, characterization of PRP products with specific focus on canine patients is needed when considering results of past, current, and future clinical applications or *in vivo* experiments. Furthermore, with such characterization data, PRPs can be selected for quantification of their associated growth factor and cytokine profiles and investigation of the effects that exogenous platelet activation has on platelet morphology, platelet release of growth factor and cytokines, and tissue healing *in vitro* and *in vivo*.¹

The objectives of the study reported here were to characterize the PRP product generated by use of several commercially available PRP-concentrating systems advertised for use on blood samples obtained from dogs. Specific aims were to quantify the platelet concentration achieved with each system, platelet capture efficiency, and leukocyte and erythrocyte concentrations or reductions in the PRP products.

Materials and Methods

Animals

Fifteen healthy dogs were recruited for the study, which was conducted at the University of Georgia. Dogs were required to weigh at least 15 kg and to be free of medical problems, other than osteoarthritis, as determined on the basis of medical history and results of a general physical examination and a CBC. Results for the CBC were required to be within respective reference ranges, with no thrombocytosis ($> 600,000$ platelets/ μL) or thrombocytopenia ($< 125,000$ platelets/ μL). Dogs with underlying immune-mediated disease were excluded. All dogs, except for 1, were not receiving any medication throughout the study period; that 1 dog received carprofen once daily throughout the study period. The study protocol and use of these dogs was approved by the Clinical Research Committee at the University of Georgia.

PRP systems

Five commercially available systems were used for PRP preparation (systems 1,^a 2,^b 3,^c 4,^d and 5^e). Four of these systems (systems 1, 2, 4, and 5) involved use of centrifugation, and 1 (system 3) was a filtration-based system.

Blood acquisition

Each dog was sedated 3 times for acquisition of blood that was used for PRP preparation; there was a 4-week interval between successive phlebotomies. Dogs were sedated for phlebotomy by IV administration of dexmedetomidine^f (0.005 mg/kg) and nalbuphine hydrochloride^g (0.5 mg/kg). Sedated dogs were placed in lateral recumbency, and blood was collected from a jugular vein.

For systems 1 and 2, blood was collected with a needle into evacuated blood tubes containing anticoagulant (sodium citrate or ACD-A) provided by the manufacturer. For system 1, there was only 1 blood tube in the kit, and it was filled in accordance with the manufacturer instructions. For system 2, 4 blood tubes (total volume, 40 mL) were used, although manufacturer instructions stated that only 2 blood tubes (total volume, 20 mL) were needed when preparing PRP from canine blood. For systems 3, 4, and 5, an 18-gauge, 2-inch catheter was inserted into a jugular vein, and blood was collected into an appropriately sized syringe containing ACD-A (variable volumes of anticoagulant and blood could be used with these systems). Blood volumes of 55, 29, and 44 mL were added to 5, 3, and 6 mL of ACD-A for systems 3, 4, and 5, respectively.

After blood sample acquisition, tubes were manually inverted several times and then placed on a blood tube rocker for 5 minutes to thoroughly mix the blood and anticoagulant. An aliquot (2 mL) of anticoagulated whole blood was then removed and added to a tube containing EDTA; that tube was maintained on a blood tube rocker to thoroughly mix the blood and

EDTA. Tubes remained on the blood tube rocker until analysis.

PRP preparation and sample processing

The blood remaining after removal of the 2-mL aliquot was processed to yield PRP by use of manufacturer-provided materials and centrifuges and in accordance with manufacturer instructions. However, for system 2, we did not use the manufacturer-provided centrifuge. Rather, a centrifuge^h with a swinging bucket rotor (radius, 16.8 cm) was used, with the acceleration and deceleration both set at 4 (scale of 1 to 10). Amount of time for each centrifugation was measured from when the centrifuge started (rather than after the rotor reached the final speed). Air temperature was 20°C. For system 5, an Hct setting of 2% was selected from the possible range of 2% to 15% on the manufacturer-provided centrifuge. This setting was chosen on the basis of preliminary data that indicated the Hct and leukocyte concentration in the PRP increased with a higher centrifuge Hct setting while the platelet concentration in the PRP did not increase. An aliquot (2 mL) of PRP was collected and placed in a blood tube with EDTA; this aliquot of PRP was handled identically to the aliquots of whole blood.

Whole blood and PRP analysis

All samples of whole blood and PRP were maintained on a blood tube rocker up to the time of sample analysis. Speed of the rocker was consistent for all samples. All samples were analyzed 15 to 60 minutes after sample collection. A CBC was performed on all whole blood and PRP samples with a hematology analyzerⁱ that used impedance technology to quantify the Hct and leukocyte, RBC, and platelet concentrations in each sample. A blood smear of each sample was used to assess platelet clumping.

Data analysis

Platelet concentration yielded by each system was quantified by dividing the platelet concentration

in the PRP by the platelet concentration in the original whole blood sample for each dog. The mean of these values for the 15 dogs was then calculated for each system. The degree of leukocyte concentration was calculated in the same manner and similarly expressed as a mean ratio for the 15 dogs for each system. Mean Hct of the PRP samples was calculated for each system. Volume of PRP produced for each dog was recorded, and the mean volume of PRP was calculated for each system. Ratio of the platelet concentration in the PRP to the leukocyte concentration in the PRP was calculated for each dog, and the mean platelet-to-leukocyte concentration ratio in the PRP was calculated for each system. Finally, the platelet capture efficiency was calculated for each dog by use of the following equation:

$$\text{Capture efficiency} = (\text{volume of PRP} \times \text{platelet concentration of PRP}) / (\text{volume of anticoagulated whole blood} \times \text{platelet concentration of anticoagulated whole blood}) \times 100$$

Mean platelet capture efficiency was calculated for each system.

Results

Animals

Age of the 15 dogs ranged from 18 months to 9 years, and body weight ranged from 17 to 45 kg. No dogs had any medical conditions, except for osteoarthritis in some dogs. No dogs were receiving medication throughout the study period, except for 1 dog that received carprofen (100 mg) daily throughout the study.

Characteristics of the systems and PRPs

The PRP systems differed substantially in the amount of blood processed, method of PRP preparation, amount of PRP produced, and platelet, leukocyte, and erythrocyte concentrations or reductions relative to concentrations in whole blood (**Tables 1 and 2**). The blood volume that was used ranged from 9 mL

Table 1—Mean \pm SD values for characteristics of whole blood samples obtained from 15 dogs between 18 months and 9 years of age with no concurrent disease, except for osteoarthritis in some dogs, and PRP products prepared by use of a variety of commercially available systems.

Variable	PRP-concentrating system*				
	1	2	3	4	5
Whole blood					
Platelet count (cells/ μ L)	207,133 \pm 37,444	226,533 \pm 40,555	249,933 \pm 37,051	260,867 \pm 47,885	252,867 \pm 39,279
Leukocyte count (cells/ μ L)	8,467 \pm 2,605	7,733 \pm 2,198	8,200 \pm 2,303	8,047 \pm 2,474	7,787 \pm 2,451
Hct (%)	42.4 \pm 3.7	39.9 \pm 2.9	43.3 \pm 3.5	42.5 \pm 0.7	41.3 \pm 4.4
PRP					
Platelet count (cells/ μ L)	169,933 \pm 3,695	743,000 \pm 301,719	452,800 \pm 185,747	1,340,667 \pm 285,520	1,035,667 \pm 514,614
Leukocyte count (cells/ μ L)	1,100 \pm 600	1,393 \pm 1,128	19,967 \pm 4,936	25,807 \pm 6,993	10,927 \pm 4,894
Hct (%)	0	0	22.2 \pm 1.6	13.4 \pm 4.0	0.06 \pm 0.2

*Five commercially available systems were used (system 1,^a 2,^b 3,^c 4,^d and 5^e); 4 of these systems (systems 1, 2, 4, and 5) involved use of centrifugation, and 1 (system 3) was a filtration-based system.

Table 2—Mean \pm SD values for characteristics of whole blood samples obtained from 15 dogs between 18 months and 9 years of age with no concurrent disease, except for osteoarthritis in some dogs, and PRP prepared by use of a variety of commercially available systems.

Variable	PRP-concentrating system*				
	1	2	3	4	5
Whole blood volume (mL)	9	34	55	29	44
PRP volume (mL)	3.3 \pm 0.41	3.0 \pm 0	6.2 \pm 0.40	4.0 \pm 0.05	2.9 \pm 0.65
Platelet ratio	0.85 \pm 0.46	3.21 \pm 0.93	1.84 \pm 0.81	5.15 \pm 0.62	3.98 \pm 1.65
Leukocyte ratio	0.14 \pm 0.07	0.17 \pm 0.10	2.49 \pm 0.44	3.27 \pm 0.50	1.40 \pm 0.45
PRP platelet-to-leukocyte ratio	173 \pm 62.5	820 \pm 520.0	23 \pm 9.6	55 \pm 17.5	129 \pm 95.3
Platelet capture efficiency (%)	30 \pm 14	25 \pm 7	20 \pm 8	68 \pm 8	28 \pm 14

Leukocyte ratio was calculated as leukocyte concentration in PRP/leukocyte concentration in anticoagulated whole blood. Platelet ratio was calculated as platelet concentration in PRP/platelet concentration in anticoagulated whole blood. Capture efficiency was calculated as (volume of PRP \times platelet concentration of PRP)/(volume of anticoagulated whole blood \times platelet concentration of anticoagulated whole blood) \times 100.

See Table 1 for remainder of key.

for system 1 to 55 mL for system 3. Mean volume of PRP ranged from 2.9 mL for system 5 to 6.2 mL for system 3. Mean degree of platelet concentration (relative to the concentration in whole blood) ranged from 0.85 for system 1 to 5.15 for system 4. Mean leukocyte reduction or concentration (relative to results for whole blood) ranged from 0.14 for system 1 to 3.27 for system 4. Mean Hct of the PRP was negligible for systems 1, 2, and 5 but was as high as 22% for system 3. Mean platelet-to-leukocyte concentration ratio in PRP ranged from 23 for system 3 to 820 for system 2. The platelet capture efficiency ranged from 20% (system 3) to 68% (system 4). Total number of times that samples were exposed to air or blood tubes were punctured after completion of blood collection was 1, 5, 1, 3, and 1 for systems 1 to 5, respectively.

Discussion

Characteristics of the PRP assessed in the present study differed for a number of factors, including the degree of platelet concentration that was achieved above that in the whole blood sample. Four systems provided a consistent but variable concentration of platelets, whereas 1 system had a mean decrease in platelet concentration in the PRP. Without an increase in platelet concentration above that in the baseline whole blood sample, some of the PRP samples did not meet the definition of being platelet-rich products. However, for the purposes of simplicity, all plasma products in the present study were referred to as PRP.

Although intuitively it might be expected that higher platelet concentrations in the PRP would be beneficial by providing a higher concentration of growth factors, this may be an erroneous assumption. In fact, increasing platelet numbers may be detrimental in that platelets can be a source of inflammatory cytokines.¹ Investigators of 1 study²⁰ found that in vitro equine tenocyte production of collagen is reduced by increasing the platelet concentration in leukocyte-reduced PRP. Similarly, the addition of increasing concentrations of platelet-derived growth factor decreases osteoinduction with demineralized bone matrix in mice, which clearly reveals that the dose-response curve is not linearly related to the concentration of a

growth factor provided by PRP.²¹ Although these are just 2 examples, results of numerous other studies have suggested that there are nonlinear dose-response curves of various cell types to different growth factors.¹ However, the authors are unaware of any such studies conducted with canine cells. As a result, the responses of specific canine cell types (chondrocytes, osteoblasts, tenocytes, ligamentocytes, and myocytes) to PRPs of variable platelet or growth factor concentrations remain unknown, and the authors urge caution regarding the assumption that a product with the highest platelet concentration is necessarily the best.

Another characteristic that differed between the PRPs in the present study was the degree of leukocyte concentration or reduction achieved with the systems. Two systems increased the leukocyte concentration, 1 system did not change the leukocyte concentration substantially, and 2 systems were leukoreductive. Accordingly, the mean platelet-to-leukocyte concentration ratios in the PRPs differed tremendously, from 23 to 820. Investigators of a seminal study⁶ found that the concentration of inflammatory cytokines in human PRP is correlated with the concentration of leukocytes in the PRP, which raises concerns that PRPs with high leukocyte numbers may be detrimental to healing. As a result, numerous studies have been conducted to evaluate the importance of leukocyte reduction and the effects of PRPs with various platelet-to-leukocyte concentration ratios. An in vivo study²² in which rabbit tendons were injected with leukocyte-rich or leukocyte-poor PRP revealed that the acute inflammatory response 5 days after injection was higher in rabbits that received the leukocyte-rich PRP. Similarly, higher concentrations of leukocytes in PRP increased expression of inflammatory cytokines by equine tendon explants.²³ Analysis of these data suggests that a leukocyte-reduced product may be most beneficial. However, when a leukocyte-poor PRP was tested with incremental increases in the platelet concentration, thereby increasing the platelet-to-leukocyte concentration ratio, increases in the platelet-to-leukocyte concentration ratio actually had a detrimental effect on collagen

synthesis of equine tenocytes.²⁰ As a result, the ideal concentration of leukocytes or the optimal ratio of platelets to leukocytes remains unknown.

Finally, the importance of erythrocytes or hemoglobin in PRP is debatable. Investigators of 1 study²⁴ evaluated the effects of leukocyte-rich PRP, leukocyte-poor PRP, platelet-poor plasma, and RBC concentrate on human synoviocytes in vitro. Those investigators found that cell death was significantly greater with leukocyte-rich PRP than with leukocyte-poor PRP, saline (0.9% NaCl) solution, and platelet-poor plasma. Cell death was even more significant with RBC concentrate. Analysis of those results suggests that only PRPs with a negligible Hct should be used for intra-articular application. However, it should be mentioned that the system that yielded the PRP with the greatest Hct in the present study has previously been assessed and been found to yield a PRP that proved efficacious in improving weight bearing in dogs with osteoarthritis when it was administered intra-articularly.⁸ Hence, it can be argued that the relevance of erythrocytes in canine PRP remains uncertain.

Although the study reported here provided good initial data on characterization of the PRP products generated by use of 5 commercially available systems, there are limitations that should be considered when interpreting the results. First, platelet counting in canine PRPs has not been validated, and it has been recommended²⁵ that hematology analyzers should be validated specifically for counting platelets in PRP. However, preliminary data, which included assessment of various sample handling techniques, anticoagulants, and 2 hematology analyzers (one that used impedance technologyⁱ and the other that used light-scatter technology^j), were acquired prior to initiation of the present study. Subjective assessments suggested that platelet clumping was minimized by gentle rocking to mix anticoagulant with the PRP samples for at least 5 minutes and preferably 15 minutes before assessment. This has been recommended by authors of the study²⁵ that validated platelet counting in PRP by use of human and bovine samples. In addition, the whole blood and PRP were treated similarly in that even though they were initially anticoagulated with sodium citrate or ACD-A, the samples were subsequently mixed with EDTA before cell counting because analysis of preliminary data suggested that platelet clumping was minimized with use of EDTA. This assertion is supported to some degree by other investigations^{26–28} in which it was found that EDTA is more effective for mitigating platelet clumping than is citrate, one of the anticoagulants used with one of the systems in the present study. Finally, all the preliminary samples that were assessed with the hematology analyzer that used light-scatter technology had mean platelet volumes that were abnormally high. Similar findings with citrate-anticoagulated samples and the same machine have been reported.²⁷ Investigators of that study²⁷ suggested that high mean platelet volume values could be attributable to nonsphering of platelets. As a result,

the hematology analyzer that relied on impedance technology and that provided mean platelet volumes within the reference range during the preliminary assessment was used for the main study. Furthermore, the manufacturer of that hematology analyzerⁱ stated that platelet counts measured with that machine were accurate up to and exceeding the platelet concentrations obtained for the PRPs in the study reported here.

Another limitation of the present study was the fact that 1 dog received an NSAID throughout the study period. Because inhibition of prostaglandin production could potentially affect platelet activation and morphology, use of an NSAID could potentially affect the ability of a system to isolate and concentrate platelets in PRP. Accordingly, results for this dog may not have been representative of results that would have been obtained had this dog not been receiving an NSAID. However, the dog received the same medication at the same dose throughout the entire study period. As a result, any effect of the NSAID on PRP yield would have remained constant for all systems assessed. Furthermore, PRP is commonly used to treat dogs that are concurrently receiving NSAIDs for musculoskeletal disease; thus, the results obtained for this dog should be clinically relevant.

Finally, it should be mentioned that we used commercially available systems, but PRP has been prepared by use of centrifugation principles with basic laboratory supplies.^{10,13} Potential advantages for use of commercially available kits are that preparation times can be shorter and less handling of the blood sample may be required. As a result, risk of contamination may be decreased. Similarly, among the commercially available systems, there was wide variability in the number of times the blood sample or PRP product was exposed to air or manipulated. Accordingly, we would hypothesize that there might be a greater risk of contamination with those commercial systems that require greater manipulation of samples. However, microbial culturing was not performed for any of the PRPs obtained. Such data should be acquired in future studies to determine whether some methods are more prone to bacterial contamination.

Analysis of the data for the present study indicated that PRP systems produced canine PRPs with differing characteristics. This is important because the efficacy of one canine PRP may differ from that of other canine PRPs, as has been concluded regarding human PRP.¹ Accordingly, broad statements about the effectiveness of PRP in canine medicine cannot be made. Rather, conclusions should be made in the context of particular PRPs and the specific tissues or medical conditions treated. We did not evaluate the clinical efficacy of any of the products tested. Hence, these data do not provide information on clinical superiority of one product over another, and the ideal characteristics of canine PRP remain uncertain. Furthermore, studies conducted to evaluate the efficacy of PRP should provide detailed data on characteristics of the PRP product used.

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Footnotes

- a. Protec PRP, PulseVet, Alpharetta, Ga.
- b. MediVet PRP, MediVet America, Nicholasville, Ky.
- c. C-PET, Pall Corp, Port Washington, NY.
- d. SmartPreP 2, Harvest Technologies, Plymouth, Mass.
- e. Angel, Arthrex Vet Systems, Naples, Fla.
- f. Dexdomitor, Zoetis Inc, Florham Park, NJ.
- g. Hospira Inc, Lake Forest, Ill.
- h. Sorvall Legend X1R centrifuge with a TX-400 rotor, Thermo Fisher Scientific, Waltham, Mass.
- i. HemaTrue, Heska Corp, Loveland, Colo.
- j. Advia 120, Siemens, Malvern, Pa.

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