

Evaluation of eicosanoid concentrations in stored units of canine packed red blood cells

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OBJECTIVE

To evaluate eicosanoid concentrations in freshly prepared canine packed RBCs (PRBCs) and to assess changes in eicosanoid concentrations in PRBC units over time during storage and under transfusion conditions.

DESIGN

Prospective study.

SAMPLE

25 plasma samples from 14 healthy Greyhounds.

PROCEDURES

Plasma samples were obtained during PRBC preparation (donation samples), and the PRBC units were then stored at 4°C until used for transfusion (≤ 21 days later; $n = 17$) or mock transfusion if expired (22 to 24 days later; 8). Immediately prior to use, 100 mL of saline (0.9% NaCl) solution was added to each unit and a pretransfusion sample was collected. A posttransfusion sample was collected after transfusion or mock transfusion. Concentrations of arachidonic acid, prostaglandin (PG) $F_{2\alpha}$, PGE_2 , PGD_2 , thromboxane B_2 , 6-keto-PGF $_{1\alpha}$, and leukotriene B_4 were measured by liquid chromatography–mass spectrometry and analyzed statistically.

RESULTS

Median arachidonic acid concentration was significantly decreased in posttransfusion samples, compared with the concentration in donation samples. Median PGF $_{2\alpha}$, 6-keto-PGF $_{1\alpha}$, and leukotriene B_4 concentrations were significantly increased in pretransfusion samples, compared with those in donation samples. Median PGF $_{2\alpha}$, thromboxane B_2 , and 6-keto-PGF $_{1\alpha}$ concentrations were significantly increased in posttransfusion samples, compared with those in pretransfusion samples. Duration of PRBC storage had significant associations with pretransfusion and posttransfusion arachidonic acid and thromboxane B_2 concentrations.

CONCLUSIONS AND CLINICAL RELEVANCE

Concentrations of several proinflammatory eicosanoids increased in PRBC units during storage, transfusion, or both. Accumulation of these products could potentially contribute to adverse transfusion reactions, and investigation of the potential association between eicosanoid concentrations in PRBCs and the incidence of transfusion reactions in dogs is warranted. (*J Am Vet Med Assoc* 2017;250:191–198)

Packed RBC transfusions are commonly used in the treatment of anemia in veterinary patients. Unfortunately, transfusion recipients are at risk of developing adverse reactions. In veterinary medicine, the incidence of transfusion reactions ranges from approximately 3% to 13%¹⁻³ in dogs receiving blood products, with febrile nonhemolytic transfusion re-

actions being the most common.¹ The incidence of transfusion reactions may actually be greater than reported in critical patients because complications may instead be attributed to the primary disease.² Pretransfusion tests such as blood typing and cross-matching are designed to assess the immunologic compatibility of blood products with recipients prior to administration. However, despite these pretransfusion tests for RBC compatibility, some recipients still develop life-threatening reactions.⁴⁻⁶ There are multiple recognized potential causes of transfusion reactions beyond those associated with RBC incompatibility, including the transfusion of fragile, damaged, or hemolyzed RBCs. Prolonged duration of storage or a suboptimal storage environment can contribute to increased RBC fragility, leading to pretransfusion and

ABBREVIATIONS

COX	Cyclooxygenase
IL	Interleukin
LOX	Lipoxygenase
LT	Leukotriene
PG	Prostaglandin
PRBC	Packed RBC
TX	Thromboxane

posttransfusion hemolysis.⁷ Recently, Patterson et al⁷ reported the deaths of 3 dogs that developed acute hemolytic reactions attributed to inappropriate storage of RBC products. In human medicine, it has been shown that the simple act of storing blood products contributes to RBC fragility and leads to the accumulation of several vasoactive and proinflammatory molecules, including cytokines (IL-1 β , IL-6, and IL-8) and arachidonic acid-derived eicosanoids.^{8,9}

Accumulation of bioactive molecules in blood products is believed to substantially increase the risk of transfusion reactions.^{8,10-12} The processing and storage of human blood products creates an environment that can lead to increased synthesis of several bioactive eicosanoids, including PGE₂,^{8,12} TXB₂,⁸ and LTB₄.⁹ Eicosanoids are signaling molecules generated following the release of arachidonic acid from the phospholipid bilayers of cells, especially platelets and cells of the immune system. Once released, arachidonic acid is enzymatically oxidized into vasoactive molecules that have multiple biological functions, including stimulation of monocyte and macrophage cytokine production, antibody synthesis, platelet aggregation, vasoconstriction, and bronchoconstriction.¹³ Because leukoreduction (removal of WBCs from blood products by filtration) is not routinely performed in veterinary medicine, most units of canine PRBCs contain substantial numbers of leukocytes and platelets that can potentially serve as sources of the arachidonic acid needed for eicosanoid biosynthesis.

Information on how the management of stored blood products from veterinary patients can influence production of bioactive molecules such as eicosanoids is lacking. The purpose of the study reported here was to evaluate eicosanoid concentrations in plasma extracted from freshly collected canine whole blood during the production of PRBC units and to determine how these variables change during the subsequent storage and transfusion of these units. Our hypotheses were that storage of PRBC units with refrigeration (4°C) would lead to an increase in eicosanoid biosynthesis and that the magnitude of the accumulation of eicosanoids would be proportional to storage duration. Additionally, we hypothesized that eicosanoid concentrations within each unit would further increase as the unit warmed toward room temperature (approx 25°C) during the time needed to complete transfusion.

Materials and Methods

Dogs and sample handling

Samples used in the study were collected from 14 healthy adult Greyhounds used as part of the Mississippi State University canine blood donor program. Health status of the dogs was established by detection of no abnormalities on physical examination; results of a CBC (including manual platelet count), serum biochemical analysis, and urinalysis within the respective reference ranges; and negative results

for *Babesia* and rickettsial serologic tests and a PCR assay for evidence of *Babesia* infection. The blood donor program donation schedule included a rotation of donor dogs with a ≥ 28 -day recovery period after each donation before another unit was collected from the same dog. The regular donation schedule was not altered for the purposes of the study, and the donor dogs did not receive any medications or treatments specifically for this project. Animal use was approved by the Mississippi State University Institutional Animal Care and Use Committee and was in compliance with the requirements for a facility accredited by the American Association for Accreditation of Laboratory Animal Care International.

A standard blood collection process was followed. Briefly, the dogs were positioned in left or right lateral recumbency, the fur overlying a jugular vein was clipped, and the skin was aseptically prepared. A 16-gauge needle was inserted into the jugular vein, and approximately 450 mL of blood was collected aseptically with negative pressure into a standard double blood-banking bag containing citrate phosphate dextrose adenine.^a

Units of PRBCs were created by separating RBCs and plasma via centrifugation at 4,200 X g and 10°C for 10 minutes. To remove the plasma following centrifugation, external pressure was applied to the blood bag, and the plasma passed via a connecting tube into an attached, empty bag for subsequent storage as fresh frozen plasma. While still containing residual plasma, the connecting tube between the 2 blood bags was sealed and removed. The plasma in the sealed connective tube was collected and stored at -20°C for later analysis. This plasma represented the initial sample (termed donation sample) for each unit of blood. Each unit of PRBCs was then stored vertically at 4°C in a dedicated blood bank refrigerator.

Each unit of PRBCs was stored in refrigeration until it was needed by a canine patient. At that time, a unit was removed from storage, infused with 100 mL of sterile saline (0.9% NaCl) solution, and mixed gently. A blood administration set, including an in-line filter, was attached to the unit and primed. Approximately 5 to 7 mL of the mixture was then collected by free flow from the tip of the administration set and centrifuged at 1,800 X g for 8 minutes at 25°C to separate the RBCs and plasma-saline components. The plasma-saline supernatant (termed the pretransfusion sample) was collected and stored at -20°C for later analysis. The recipient was then administered the unit of PRBCs over a 3- to 6-hour period at the discretion of the attending clinician. The unit of PRBCs was held at room temperature for the duration of the transfusion. On completion of the transfusion, the PRBCs remaining within the unit were collected and centrifuged by the same procedure used for the pretransfusion sample. The supernatant (termed the posttransfusion sample) was collected and stored at -20°C for later analysis. If a unit of PRBCs was not used within the institution's established storage life

of 21 days, it was considered to be expired. Within 1 to 3 days after expiration (maximum storage duration, 24 days), each expired unit was infused with saline solution and, although the unit was not administered to a live recipient, mock pretransfusion and posttransfusion (5 hours after removal from refrigeration) samples were collected, centrifuged, and stored as described for the units that were used clinically.

Eicosanoid concentration measurements

Concentrations of arachidonic acid, PGF_{2α}, PGE₂, PGD₂, TXB₂ (a stable metabolite of TXA₂), 6-keto-PGF_{1α}, (a stable, hydrolyzed product of PGI₂ [ie, prostacyclin]), and LTB₄ in plasma or plasma-saline mixtures were analyzed via liquid chromatography-mass spectrometry with previously established techniques.¹⁴ Briefly, thawed canine plasma containing deuterated internal standards (*d*₄-8-iso PGF_{2α}, *d*₄-LTB₄, and *d*₈-arachidonic acid) were extracted by solid-phase methods with C18 silica-based sorbent columns.^b After filtration, a 10-μL aliquot of the resolubilized lipids was injected onto an ultraperformance liquid chromatography C18 column (1.7 μm; 100 X 2.1-mm internal diameter).^c Analytes were eluted from the column with a gradient protocol. The column eluate was directed into a mass spectrometer.^d

Prostaglandins and LTs were quantified by measuring the area under each chromatographic peak and comparing it with the area under the chromatographic peak for the appropriate internal standard. The eicosanoid amounts were then corrected by a predetermined instrument response factor that represented the ratio of the mass spectrometric signal obtained from equimolar quantities of native eicosanoid versus the appropriate deuterated internal standard. Finally, PG and LT amounts were normalized to the volume of plasma used for analysis and expressed as a concentration (pmol/mL of plasma). The estimated limits of detection for the PGs and LTs by this analytic method were between 0.01 and 0.1 nmol on-column.

Statistical analysis

To determine the effect of storage duration on each outcome measured for (actual or mock) pretransfusion and posttransfusion samples, a mixed-model analysis was conducted with a statistical program.^c The day of blood (and donation sample) collection was considered day 0. For each molecule quantified, a model was fit with fixed effects of storage duration, sample point, storage duration by sample point interaction, and molecule concentration measured at donation as a covariate. Unit within dog was included as a random effect with variance component covariance structure. The distribution of the conditional residuals was evaluated for each molecule to determine the appropriateness of the statistical model. The relationship between storage duration and model-predicted concentrations of each molecule was demonstrated graphically by plotting regression lines for pretransfusion and posttransfusion sample points.

To compare the measured outcomes at each of the sample points, including the donation sample, a similar mixed model was developed but with only sample point as a fixed effect. Storage duration was not included in the model as it could not have an effect on the measures taken with the donation sample. The distribution of the conditional residuals was evaluated for each molecule and found to be nonrandomly distributed when plotted against the predicted values. The frequency distributions of the residuals were generally nonnormal as well. Consequently, a method similar to the nonparametric Friedman test was conducted instead. For each molecule evaluated, the data were first ranked for each unit of blood. An ANOVA was then conducted on the ranked data with unit and sample point as fixed effects. Differences in least squares means with Bonferroni adjustment for multiple comparisons were determined for molecules with a significant sample point effect. An α of 0.05 was used to determine statistical significance for all methods.

Results

Twenty-five units of PRBCs collected from the 14 donor Greyhounds were used in the study. There were no adverse events detected for donors during or after blood collection. Four donors provided 1 unit each, 9 donors provided 2 units each, and 1 donor provided 3 units over the 6-month study period. The units were stored with refrigeration for 1 to 24 days, with a median storage duration of 16 days (range, 1 to 24 days; **Figure 1**). Seventeen of these units were used clinically, and 8 were expired units subjected to mock transfusion conditions. The plasma (donation) and plasma-saline supernatant (pretransfusion and posttransfusion) samples were stored at -20°C for 2 to 6 months before analysis was performed. All transfusions were administered over 3 to 6 hours after each unit was removed from refrigeration.

The plasma concentrations of arachidonic acid and eicosanoids in the 25 units of PRBCs at the time of donation, after storage (pretransfusion), and after transfusion (posttransfusion) were summarized (**Table 1**). The median arachidonic acid concentration in the pretransfusion samples did not differ significantly ($P = 0.495$) from that in donation samples. However, the posttransfusion arachidonic acid concentration was significantly ($P = 0.014$) decreased, compared with that in donation samples. There was no significant difference in arachidonic acid concentration between the pretransfusion and posttransfusion samples.

The median PGE₂ and PGD₂ concentrations in pretransfusion and posttransfusion samples did not differ from those in donation samples ($P = 1.00$ for all comparisons). There was a significant increase in median PGF_{2α} concentrations in pretransfusion ($P = 0.018$) and posttransfusion ($P < 0.001$) samples, compared with that in donation samples. Additionally, there was a significant ($P < 0.001$) increase in PGF_{2α} concentration between the pretransfusion and posttransfusion samples.

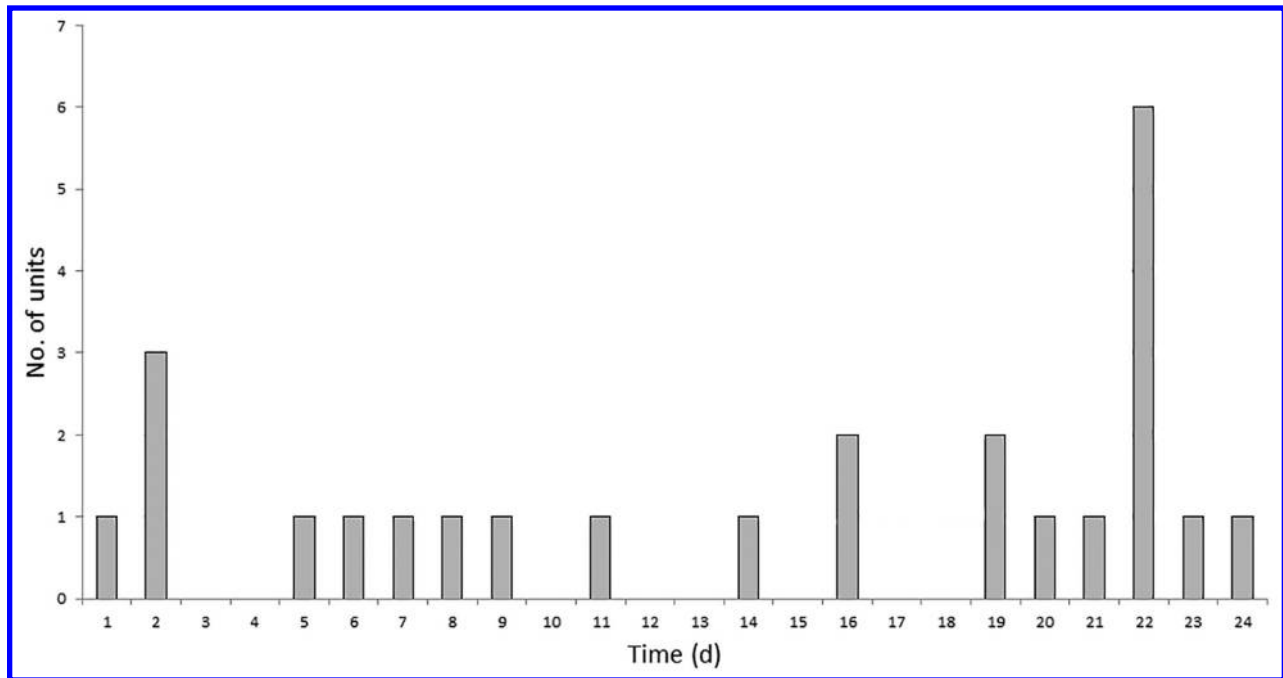


Figure 1—Storage duration for 25 units of PRBCs from 14 healthy Greyhounds in a study to evaluate eicosanoid concentrations in plasma obtained during preparation of PRBCs and to assess changes in these eicosanoid concentrations in units of PRBCs over time in storage at 4°C and under transfusion conditions. Day 0 was the day of blood collection. Units stored for 1 to 21 days ($n = 17$) were used for transfusions in clinical patients, whereas those stored for 22 to 24 days (8) were considered expired according to the hospital's standard protocols and were subjected to mock transfusion conditions for study purposes.

Table 1—Median (range) concentrations of arachidonic acid, $\text{PGF}_{2\alpha}$, PGE_2 , PGD_2 , TXB_2 , 6-keto- $\text{PGF}_{1\alpha}$, and LTB_4 in units of PRBCs collected from 14 healthy Greyhounds in a study to evaluate eicosanoid concentrations in plasma obtained during preparation of canine PRBCs and to assess changes in these eicosanoid concentrations in units of PRBCs over time during storage at 4°C and under transfusion conditions.

Molecule	Donation	Pretransfusion	Posttransfusion
Arachidonic acid (ng/mL)	4,566 (0–45,892) ^a	2,707 (0–21,725) ^{a,b}	1,368 (0–11,563) ^{b,c}
PGE_2 (ng/mL)	0.0 (0.0–11.9) ^a	0.0 (0.0–54.4) ^a	0.0 (11.1) ^a
PGD_2 (ng/mL)	0.0 (0.0–40.1) ^a	0.0 (0.0–163.7) ^a	0.0 (0.0–7.4) ^a
$\text{PGF}_{2\alpha}$ (ng/mL)	1.0 (0.0–6.6) ^a	3.0 (0.0–11.5) ^b	11.3 (0.0–73.5) ^c
TXB_2 (ng/mL)	0.0 (0.0–15.1) ^a	1.6 (0.0–24.6) ^a	2.2 (0.0–78) ^b
6-keto- $\text{PGF}_{1\alpha}$ (ng/mL)	11.7 (0.0–17.2) ^a	32.7 (11.3–32.7) ^b	51.6 (10.6–155.9) ^c
LTB_4 (ng/mL)	0.3 (0.0–9.3) ^a	1.5 (0.2–124.1) ^b	2.6 (0.0–50.1) ^b

Donation samples were collected from fresh plasma obtained during preparation of PRBCs. The PRBC units were administered to patients when needed up to 21 days after collection; PRBCs > 21 days old were considered expired and were not used clinically, but were subjected to mock-transfusion conditions within 3 days after expiration for study purposes. Pretransfusion samples were collected after storage and immediately prior to (actual [$n = 17$] or mock [8]) transfusion; 100 mL of sterile saline (0.9% NaCl) solution was added to the unit, and after gentle mixing, 5 to 7 mL of the PRBC-saline mixture was collected. Following centrifugation, the plasma-saline supernatant was collected and used for measurement of eicosanoid concentrations. Posttransfusion samples were obtained by harvesting PRBC-saline mixture remaining in the unit at the end of transfusion (approx 3 to 6 hours at room temperature) and processing as described for pretransfusion samples. The posttransfusion samples from expired PRBC units were collected in the same manner 5 hours after the unit was removed from storage and infused with saline solution.

^{a-c}Within a row, values with different superscript letters are significantly ($P < 0.05$) different.

Although the median concentration of TXB_2 did not differ ($P = 1.00$) between donation and pretransfusion samples, the concentration was significantly increased in posttransfusion samples, compared with the values for donation samples ($P = 0.007$) and pretransfusion

samples ($P = 0.024$). Compared with results for donation samples, there were significant increases in median 6-keto- $\text{PGF}_{1\alpha}$ concentration in pretransfusion ($P < 0.001$) and posttransfusion ($P < 0.001$) samples. Additionally, the 6-keto- $\text{PGF}_{1\alpha}$ concentration was significantly ($P =$

0.001) increased in posttransfusion samples, compared with that in pretransfusion samples.

Compared with results for donation samples, there was a significant ($P = 0.001$ for both comparisons) in-

crease in median LTB_4 concentration in pretransfusion and posttransfusion samples. There was no significant ($P = 1.00$) difference in LTB_4 concentration between the pretransfusion and posttransfusion samples.

When results for all pretransfusion and posttransfusion samples were combined, the duration of PRBC storage had a significant ($P = 0.022$) association with TXB_2 concentrations (Figure 2). Additionally, the duration of storage by sample interaction was significant ($P = 0.010$) for arachidonic acid concentration (ie, the duration of storage effect was significant only when considered concurrently with pretransfusion or posttransfusion sample values). The duration of storage did not have a significant effect on the concentrations of any of the remaining eicosanoids.

Discussion

In the present study, refrigerated storage of canine PRBC units led to the accumulation of several eicosanoids, similar to findings reported for stored human blood products.^{8,11,12} Additionally, our results showed that the concentrations of several of the eicosanoids measured in this study increased inside the PRBC units at room temperature over the time required to administer a transfusion.

Blood cells, including platelets, do not store eicosanoids. Rather, eicosanoids are synthesized in situ from arachidonic acid, which is released from phospholipid membranes through the action of phospholipase A_2 .¹³ Phospholipase A_2 can be activated by several different stimuli, including hormones, cytokines, mechanical injury,¹⁵ and a decrease in temperature,¹⁶ all of which have the potential to influence cells inside a unit of blood during storage and transfusion. Once mobilized, free arachidonic acid is metabolized by the COX, LOX, and cytochrome P450 enzymatic pathways. In the COX pathway, arachidonic acid is converted by COX-1 or COX-2 enzymes into several PGs and TXs. Similarly, in the LOX pathway, arachidonic acid is converted by LOX enzymes (5-LOX, 12-LOX, and 15-LOX) into several LTs.¹³ In our study, compared with findings for initial donation samples, the concentration of arachidonic acid had decreased by the completion of transfusion, suggesting that the environment in-

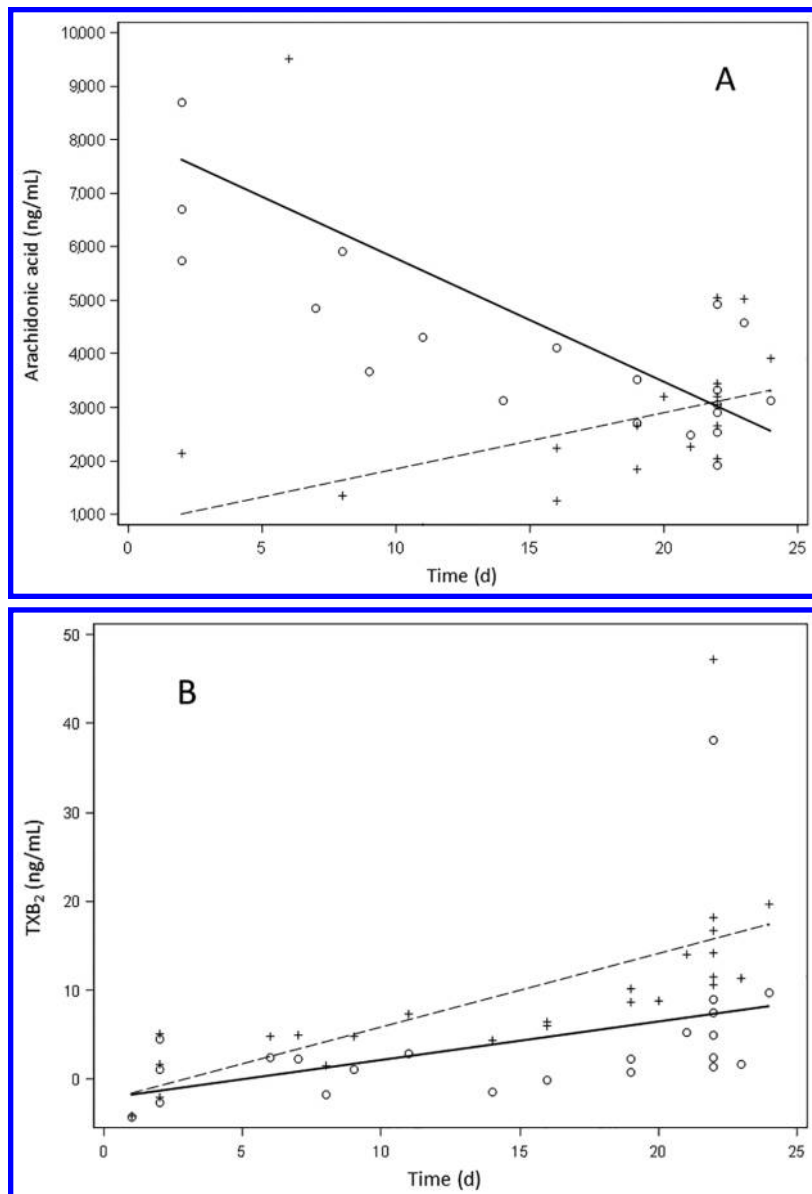


Figure 2—Regression lines depicting the relationships between duration of storage at 4°C and model-predicted concentrations of arachidonic acid (A) or TXB_2 (B) in 25 units of canine PRBCs in pretransfusion (solid lines and circles) and posttransfusion (dashed lines and crosses) samples. Pretransfusion samples were collected after storage and immediately prior to (actual or mock) transfusion; 100 mL of sterile saline (0.9% NaCl) solution was added to the unit, and after gentle mixing, 5 to 7 mL of the PRBC-saline mixture was collected. Following centrifugation, the plasma-saline supernatant was collected and frozen at -20°C until use for measurement of eicosanoid concentrations. Posttransfusion samples were obtained by harvesting PRBC-saline mixture remaining in the unit at the end of transfusion (approx 3 to 6 hours at room temperature) and processing as described for pretransfusion samples. The posttransfusion samples from expired PRBC units were collected in the same manner 5 hours after the unit was removed from storage and infused with saline solution. A—As storage duration increased, the concentration of arachidonic acid in pretransfusion samples decreased; however, in posttransfusion samples, the concentration of arachidonic acid increased as duration of storage increased. B—In pretransfusion and posttransfusion samples, increased duration of unit storage was associated with increased concentrations of TXB_2 .

side the unit can enhance the release and enzymatic consumption of arachidonic acid. Even though we did not measure all of the eicosanoids that could potentially be produced by all enzymatic pathways (eg, the cytochrome P450 pathway was not evaluated in this study), significant increases in concentrations of several products (PGF_{2α}, 6-keto-PGF_{1α}, and TXB₂) suggested that the COX pathway greatly contributes to the metabolism of mobilized arachidonic acid in units of PRBCs.

Although erythrocytes have the ability to produce eicosanoids,^{17,18} platelets and leukocytes are the primary producers of these biologically active molecules.^{13,19} Platelets express COX-1 as their primary COX isoform and are the primary source of TX synthesis in the circulation. Nucleated cells, including leukocytes, express COX-2 as their primary COX isoform and are responsible for the synthesis of PGs, including PGI₂, PGD₂, PGE₂, and PGF_{2α}.^{13,19} In our study, TXB₂ concentrations were increased in pretransfusion and posttransfusion samples, compared with that in donation samples, presumably as a result of COX-1 enzyme activity in platelets. Concentrations of 6-keto-PGF_{1α} (a stable PGI₂ metabolite) and PGF_{2α} were increased in pretransfusion and posttransfusion samples, compared with those in donation samples, and in posttransfusion samples, compared with those in pretransfusion samples. These PGs were presumably derived via COX-2 enzyme activity in leukocytes. Taken together, these data suggested that mobilized arachidonic acid was metabolized by both COX isoforms. However, the increases in 6-keto-PGF_{1α} and PGF_{2α} were substantially higher than that of TXB₂, possibly indicating that the COX-2 enzyme pathway was predominant.

Febrile nonhemolytic transfusion reactions are the most common adverse transfusion reactions in human and veterinary medicine. In people, transfusion with platelet products is most commonly associated with this type of reaction, although it can develop in response to any blood product that contains platelets or leukocytes.^{6,20,21} Prestorage leukoreduction with a leukoreduction filter has been associated with a decrease in the frequency of febrile nonhemolytic transfusion reactions in human patients.²²⁻²⁴ Additionally, the prestorage use of a leukoreduction filter decreases the concentration of PGE₂ and TXB₂ in units of human PRBCs that have been stored for 21 days¹²; however, it is unknown whether the decrease in the frequency of such reactions following prestorage leukoreduction is a direct result of a decrease in eicosanoid concentrations. It is also unknown whether the removal of platelets and leukocytes via leukoreduction filters prior to storage would influence the concentration of eicosanoids in units of canine PRBCs.

Several proinflammatory cytokines, such as IL-1β, IL-6, IL-8, and tumor necrosis factor-α, accumulate in units of human PRBCs during storage and have also been implicated in contributing to febrile nonhemolytic transfusion reactions in people.¹¹ Interleukin-8

(also called chemokine [C-X-C motif] ligand 8) is a powerful chemoattractant of neutrophils and capable of stimulating the immune system by enhancing cytokine production and immune cell activation.¹⁰ The infusion of a blood product with a high concentration of IL-8 could thus exacerbate a transfusion reaction in the recipient.²³ There is a time-dependent accumulation of IL-8 during storage of canine PRBCs, similar to that described for human PRBCs, but IL-8 concentrations can be attenuated by prestorage leukoreduction.¹⁰ In human medicine, TXA₂ has been shown to increase IL-8 mRNA expression and protein production in endothelial cells,^{25,26} suggesting that an increase in this eicosanoid in a unit of PRBCs could potentially also contribute to IL-8 concentrations during storage and transfusion.

Although our study revealed that concentrations of multiple eicosanoids increase in units of canine PRBCs during storage, transfusion, or both, it is still unknown whether or how these accumulated molecules affect recipients. The administration of high amounts of eicosanoids via transfusion has the potential to influence or enhance several physiologic responses within the recipient. For example, TXA₂ is known to enhance platelet activation as well as to trigger vasoconstriction and bronchoconstriction, and thus, transfusion of a blood product containing a high concentration of this product has the potential to enhance hemostatic pathway reactions, especially in hypercoagulable patients. It should also be considered that PGI₂, which has physiologic effects opposite to those of TXA₂, including vasodilation, bronchodilation, and inhibition of platelet aggregation, could also adversely influence a transfusion recipient. Acute hemolytic transfusion reactions caused by RBC incompatibility are associated with the binding of the recipient's preexisting antibodies to antigens on erythrocytes, leading to cell lysis and intravascular hemolysis. Hypotension and poor renal blood flow are common clinical findings in patients with acute hemolytic transfusion reactions⁵ and could be associated with increased circulating concentrations of PGI₂. In anesthetized dogs, bolus injections of PGI₂ (1 to 100 ng/kg [0.45 to 45.5 ng/lb]) created a dose-dependent increase in arterial blood flow and splenic diameter.²⁷ The lowest concentration of this eicosanoid that caused significant vasodilation was 2 ng/kg (0.91 ng/lb). The typical body weight of dogs in that study²⁷ was 14 kg (30.8 lb); thus, a total dose of 28 ng was associated with this response. In the present study, the median concentration of the PGI₂ metabolite 6-keto-PGF_{1α} in pretransfusion samples was 32.7 ng/mL. The hemodynamic changes observed in the experimental study²⁷ in dogs were temporary, starting < 1 minute after administration, with all variables returning to the preadministration values within 5 minutes after injection. However, during a typical PRBC transfusion, eicosanoids in the product are delivered by continuous infusion over a period of hours rather than a single bolus injection. Compared with a single injection of prostacyclin under experimental conditions, it

is unknown how transfusion recipients will respond to a continuous infusion of prostacyclin-containing blood products in a clinical setting.

Results of some studies²⁷⁻³⁰ indicate that the duration of human PRBC storage prior to transfusion has a significant effect on posttransfusion morbidity and mortality rates. However, this association is controversial, and other studies^{31,32} have not found a difference in survival rates of patients receiving transfusions of fresh whole blood versus those receiving stored units of PRBCs. One large retrospective study¹ of dogs found that longer duration of PRBC unit storage prior to transfusion is a negative risk factor for survival of dogs with hemolysis. Additionally, dogs with pneumonia that received blood products stored for 42 days had a greater mortality rate, compared with that of dogs that received blood products stored for 7 days.³³ One proposed explanation for the described associations could be an increased concentration of monocyte chemoattractant protein-1, which accumulates in units of PRBCs during storage.³⁴ Blood products that contain erythrocytes usually have a standard storage duration of 28 to 35 days, and storage of units for longer than this time could influence cell viability.⁷ The maximum storage time in our study, 24 days, was shorter than that recommended in some transfusion protocols. Use of a 21-day expiration date for stored PRBCs is a standard practice in our hospital intended to minimize any adverse reaction associated with a transfusion. However, even with a storage time that is shorter than is used in many veterinary blood-banking programs, the present study found significant changes in the median concentrations of many eicosanoids, and we consider it likely that longer storage durations would be associated with greater changes in eicosanoid concentrations.

Our results indicated that the duration of PRBC unit storage significantly influenced the concentrations of arachidonic acid. One explanation for this could be the health of cells in units of PRBCs. The longer the units are stored, the greater the likelihood of decreased cell viability, increasing the possibility of the release of arachidonic acid and enzymatic conversion to eicosanoids. Additionally, during the transfusion, when units of PRBCs are warmed to room temperature, enzymatic activity is expected to increase, allowing for a greater release of arachidonic acid via phospholipase A₂ activity and more efficient conversion to eicosanoids by the pathway-specific enzymes. However, the exact mechanisms associated with the change in arachidonic acid during storage and transfusion are unknown. It is possible that the addition of an RBC preservative solution to the units can improve the viability of the erythrocytes and minimize the synthesis of PGs and LTs. The blood bags used in our study did not contain an erythrocyte preservative, and this could have contributed to the observed increases in some eicosanoid concentrations.

In our study, the duration of storage also influenced the concentration of TXB₂, similar to results of a previous study¹² performed with human blood products that found an increase in both PGE₂ and

TXB₂ concentrations during storage. In that study,¹² units of PRBCs were stored for 49 days, which is a considerably longer duration than the units assessed in the present study. It is possible that, with a duration of storage that was longer than the median of 16 days for samples used in the present study, we might have observed an effect of storage time on the concentrations of additional eicosanoids. The duration of unit storage in our study was dependent on the needs of clinical patients, which created an unequal distribution of storage durations over the study period. For example, 3 units were stored for only 2 days, whereas 6 units were stored for 22 days. We cannot rule out the possibility that, with a larger sample size, a longer storage duration, and an equal distribution of storage durations, the duration of storage would have had an effect on other eicosanoid concentrations.

Even under ideal storage and handling conditions, units of PRBCs are subject to a number of factors that could affect the production of proinflammatory molecules such as eicosanoids. Of all the eicosanoids evaluated in our study, TXB₂, PGF_{2 α} , and 6-keto-PGF_{1 α} had the greatest change in concentration from the time of collection until the completion of the transfusion. For all 3 eicosanoids, concentrations were significantly greater at the completion of the transfusion than after the storage period (immediately prior to transfusion). One of the main environmental differences between the initiation and completion of a transfusion is the change in temperature from 4°C during refrigerated storage to room temperature during transfusion. Some of the enzymes that produce eicosanoids could potentially be more efficient at temperatures > 4°C. Additionally, mechanical disruption and stress of transfused cells associated with centrifugation, kinked or narrow intravenous tubing, or clogged filters may cause shearing injury to cells, leading to hemolysis and release of arachidonic acid for eicosanoid biosynthesis. However, in a different population of cells, a similar finding occurred when mechanical stress and stimulation was applied to human cells, demonstrating an increase in the synthesis of PGI₂ and TXB₂ under such conditions.³⁵

In our study, several eicosanoids, including TXB₂, PGF_{2 α} , 6-keto-PGF_{1 α} , and LTB₄, were found to accumulate in canine PRBC units during refrigerated storage, transfusion at room temperature, or both. Further investigation of the relationship between eicosanoid concentrations in stored blood products and the incidence of transfusion reactions in recipient dogs is warranted.

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Footnotes

- a. Teruflex Blood Bag System, 450 mL, CPDA-1, Terumo Corp, Tokyo, Japan.

- b. HyperSep Retain PEP 60 mg, 1 mL, Thermo Fisher Scientific, Waltham, Mass.
- c. Acquity UPLC BEH C18 column, Waters Corp, Milford, Mass.
- d. Thermo Quantum Access Max, Thermo Fisher Scientific, Waltham, Mass.
- e. SAS, version 9.3, SAS Institute Inc, Cary, NC.

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