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Coagulation Factor and Hemostatic Protein Content of Canine Plasma after Storage of Whole Blood at Ambient Temperature

J.E. Walton, A.S. Hale, M.B. Brooks, A.K. Boag, W. Barnett, and R. Dean

Background: Standard practice in canine blood banking is to produce fresh frozen plasma (FFP) by separating and freezing plasma produced from blood within 8 hours of collection. Within canine blood donation programs, this can limit the number of units collected.

Hypothesis/Objectives: The aim was to compare the coagulation factor and hemostatic protein content (CF&HPC) of plasma produced from blood stored at ambient temperature for 8, 12, and 24 hours. Another aim was to compare the CF&HPC between Greyhound types and other breeds.

Animals: None.

Methods: In vitro study. A convenience sample of 58 units of canine blood from a blood donor pool was processed to prepare and freeze plasma 8, 12, or 24 hours following collection.

Results: Regardless of time of processing, the units contained therapeutic CF&HPC. Frozen plasma prepared after 24 hours had significantly higher factor VIII ($P = .014$) and factor X ($P = .03$) when compared with the frozen plasma prepared at 8 hours. Factor X ($P < .01$), fibrinogen ($P < .01$), and vWF ($P = .04$) were significantly lower in plasma collected from Greyhound types than in plasma collected from other breeds.

Conclusions and Clinical Importance: Storing whole blood for up to 24 hours is a suitable method for producing FFP. Lower values for some coagulation factors and hemostatic proteins in plasma produced from Greyhound types would not preclude these dogs as FFP donors.

Key words: Factor VIII; Hemostasis; Transfusion; von Willebrand factor.

Standard operating procedures for preparation of transfusion products are continually evolving in human transfusion medicine. It is increasingly becoming standard practice internationally to store whole blood for up to 24 hours before processing (previous limit 8 hours).[1] The United Kingdom National Blood Service Blood and Transplant (NHS BT) has been processing in this way since 2007—and terms all plasma processed within 24 hours as fresh frozen plasma (FFP).[2]

The American Association of Blood Banking still differentiates plasma frozen within 8 hours of phlebotomy, defined as FFP, from plasma frozen within 24 hours of phlebotomy, defined as FP24. However, the clinical indications for FFP and FP24 are generally the same.[3]

Processing of whole blood into packed red cells and FFP within 6–8 hours of collection has been the generally accepted practice in veterinary medicine since the inception of canine blood banking in the 1970s. This followed human blood banking practice at that time[1,2]; this timing has not been reviewed or critically evaluated in the veterinary field of blood banking despite the change in the guidance in human transfusion medicine.

Veterinary studies investigating the stability of plasma samples destined for hemostasis testing[4,5] and veterinary studies looking at the effect of storage conditions and time on coagulation factor and hemostatic protein content (CF&HPC) in stored plasma[5–8] indicate that the most labile coagulation factors and hemostatic proteins (factor VIII and vWF) are stable in canine plasma for longer periods than 8 hours.

Producing blood components from canine whole blood that has been stored for 24 hours would offer more operational flexibility and efficiency for veterinary blood banks. It would increase the geographic area that donors could be recruited from in relation to the site of processing and increase the number of donors that could donate at collection sessions. This, in turn, would mean more available units and more cost-effective products to the end user. However, it is unknown if increasing preprocessing storage time would compromise the use of these units as a source of coagulation factors and hemostatic proteins.

Abbreviations:

APTT	activated partial thromboplastin time
AT	antithrombin activity
CF&HPC	coagulation factor and hemostatic protein content
FFP	fresh frozen plasma
NHS BT	National Health Service Blood and Transplant (UK)
NSD	no significant difference
PT	prothrombin time
VWF	von Willebrand factor

From the Pet Blood Bank UK, Loughborough Technology Centre, Loughborough, UK (Walton, Barnett); The Platelet Farm, Gregory, MI (Hale); the Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY (Brooks); the Vets Now, Dunfermline, UK (Boag); and the Centre for Evidence-based Veterinary Medicine, School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK (Dean). *An abstract of this work was presented at the 54th Annual Congress of the British Small Animal Veterinary Association, Birmingham, UK, April 2012.*

Corresponding author: J.E. Walton, BVM&S MRCVS, Pet Blood Bank UK, Loughborough Technology Centre, Epinal Way, Loughborough, LE11 3GE UK;
e-mail: jenny.walton@petbloodbankuk.org.

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The majority of canine blood donor programs collect blood from donors weighing 25 kg or more. Greyhound types are often overrepresented as donors because of their availability, conformation, size, nature, and physiology.[9] Previous reports indicate that Greyhounds may, as a breed, have lower levels of some coagulation factors.[10,11] Extending the preprocessing storage times might exacerbate this concern when using Greyhound types as donors.

The aim of this study was to compare the CF&HPC of plasma prepared from different units of whole blood processed to frozen end product at one of three time intervals, 8, 12, and 24 hours postcollection. The second aim was to compare the CF&HPC of plasma produced from Greyhound types with the CF&HPC of plasma produced from other breeds of dog.

Methods

Donors

A convenience sample of 58 units of canine whole blood was selected from a total of 147 units collected by a community-based voluntary blood bank in the United Kingdom across 13 collection sessions between July 2010 and October 2010. All donors had to be 1–8 years of age, >25 kg in body weight, fully vaccinated, have no history of travel outside the United Kingdom and Ireland, and be fit and well at the time of donation.

Donors were classed as "Greyhound types", which included Greyhounds and Greyhound crosses and "other breeds." All dogs were either client-owned or kenneled retired racing Greyhounds awaiting rehoming.

Units

Blood was collected by caudal jugular venipuncture according to the participating blood bank's standard operating procedure. Donor dogs were not sedated; 450 mL (±10%) of whole blood was collected by gravity flow into a vacuum-sealed bag[a] containing 63 mL of CPD anticoagulant. Each unit was collected in <7 minutes. All units were transported and stored at ambient room temperature (18–25°C) before processing. Only units with no visible evidence of clots or marked lipemia were included in this study. One member of laboratory staff performed the processing of all units.

Sample Preparation and Storage

Units were centrifuged[b] according to the participating blood bank's standard operating procedure. The processing temperature setting was 20 ± 2°C and the spin settings used were 4207 × g. After centrifugation, the plasma component was immediately separated from the packed red blood cells using a Terumo plasma extractor. Aliquots of 2 mL of plasma were prepared from each unit's processing lines. Twenty aliquots (10 of each breed group) were frozen between 6.8 and 8.1 hours postcollection. Eighteen aliquots (10 Greyhound types and 8 other-breed group) were frozen between 10.6 and 12 hours postcollection. Twenty aliquots (10 of each breed group) were frozen between 23.3 and 24.5 hours postcollection. All aliquots were frozen at −80°C[c] until transport to the laboratory. For transfer to the diagnostic laboratory, aliquots were packaged and transported at −80°C on dry ice by specialist courier. On arrival, frozen status was confirmed and samples were stored at −70°C until thawing at 37°C immediately before assay. Assay was performed as a batch in January 2011 (3–6 months after sample collection).

Laboratory Assays

The coagulant activities of factors II, V, VII, VIII, IX, and X, the factor IIa inhibitory activity of antithrombin, von Willebrand factor concentration (VWF : Ag), and clottable (Clauss) fibrinogen were determined for all plasma samples.

The intrinsic factor assays (factors VIII and IX) were performed in a modified one-stage activated partial thromboplastin time (APTT) technique using a semiautomated clot detection instrument (ST4[d]) and canine congenital factor VIII and factor IX deficient substrate plasmas, as previously described.[7,12] Coagulant activity assays for factors II, V, VII, and X were performed in a modified one-stage prothrombin time (PT) technique using an automated clot detection instrument (STACompact[d]), a rabbit brain thromboplastin reagent,[e] and human substrate deficient plasmas (factors II, V, and VII) or an adsorbed, artificially depleted bovine plasma (factor X activity).[12,13] Plasma antithrombin activity (AT) was measured using a synthetic chromogenic substrate kit (Stachrom AT III[d]) according to the manufacturer's recommendations for assay incubation, activation, and instrumentation. Fibrinogen was measured in an automated coagulation instrument via Clauss method[12] using a 100 NIH U/mL human thrombin reagent (Fibrinogen[d]) and VWF : Ag was measured using an ELISA, configured with monoclonal anticanine vWF antibodies.[14]

The standard curves for all assays were derived from dilutions of pooled canine plasma (prepared from 20 healthy dogs, then stored in single-use aliquots at −70°C). The results of coagulant activity assays, AT, and vWF : Ag were reported as a percentage of the canine standard that had an assigned value of 100% activity or concentration. The fibrinogen content of the canine standard (mg/dL) was measured in a multispecies fibrinogen ELISA.[15]

The study was undertaken in compliance with standard operating procedure of the blood bank, which is licensed by the national regulatory body. The study was approved by the Ethics committee at the School of Veterinary Medicine and Science, The University of Nottingham.

Statistical Analysis

Descriptive and inferential statistical analysis was undertaken.[f] The data were tested for normality and found to be nonnormally distributed; therefore, the median and range are reported for all CF&HPC. Frequencies were reported as absolute numbers followed by percentages. The Kruskal–Wallis test was used to compare the CF&HPC of the aliquots at the different time points followed by posthoc comparisons using the Mann–Whitney *U*-test (where indicated). Mann–Whitney *U*-analysis was used to compare the CF&HPC between breed groups. A *P*-value of <.05 was considered significant.

Results

Donors

The Greyhound type group consisted of 29 greyhounds and 1 greyhound cross. Fourteen breeds represented in the other breed group were:

Labradors (7), Flat Coat Retrievers (4), Golden Retrievers (2), German Shepherd Dogs (2), Old English Sheepdogs (2), Weimeraners (2), and St Bernards

(2) and 1 of 7 other breeds. There were 33 males and 25 females. Seventy-nine percent of the donors were neutered. The median age was 5 years (range 1–8) The CF&HPC varied at different time points (Table 1).

The Kruskal–Wallis test demonstrated significant differences between duration of time before freezing only in factors VIII ($P = .018$) and X ($P = .005$). Mann–Whitney *U*-analysis demonstrated that frozen plasma prepared after 24 hours had significantly higher factor VIII ($P = .014$), and factor X ($P = .03$) when compared with the frozen plasma prepared at 8 hours. Factor VIII ($P = .026$) and factor X ($P = .031$) were also significantly higher in the frozen plasma prepared at 24 hours than in the frozen plasma prepared at 12 hours.

Factor VIII had the greatest range between all donor units and time points with 31–225% factor VIII content compared to the canine standard.

Factor X, fibrinogen, and vWF were significantly lower in plasma collected from Greyhound types compared to plasma collected from other breeds across all time points (Table 2).

Table 1. Coagulation factor and hemostatic protein (CF&HPC) content of canine frozen plasma samples prepared from 450 mL whole blood units at 3 different time points after collection—8, 12, and 24 hours.

CF&HPC[a]	Time (Hours)	N	Median	Range
II (%)	8	20	128	80–195
	12	18	137	84–190
	24	20	128	103–195
V (%)	8	20	137	82–183
	12	18	119.5	86–150
	24	20	136	75–198
VII (%)	8	20	107.5	43–185
	12	18	104	71–145
	24	20	115	59–149
VIII (%)	8	20	65.5	31–121
	12	18	68.5	44–225
	24	20	86.5	42–170
IX (%)	8	20	97.5	62–136
	12	18	109	43–157
	24	20	96	48–159
X (%)	8	20	97.5	82–143
	12	18	100	81–121
	24	20	110	73–143
vWF (%)	8	20	97.8	44–172
	12	18	100.5	64–146
	24	20	87.6	63–141
Antithrombin (%)	8	20	116	104–143
	12	18	122	103–146
	24	20	126.5	108–145
Fibrinogen (mg/dL)	8	20	282.5	161–512
	12	18	288	196–577
	24	20	274	191–491

[a]Units of measurement are recorded as a % of a canine standard that had an assigned value of 100% for all coagulation factors, antithrombin, and vWF : Ag, then mg/dL for fibrinogen.

Table 2. Coagulation factor and hemostatic protein content (CF&HPC) of frozen plasma samples prepared across all time points from Greyhound types (GH) compared to the CF&HPC from other breeds (Other).

CF&HPC[a]	Breed	N	Median	Range	Difference
II (%)	GH	30	126	82–184	.141
	Other	28	133.5	80–195	
V (%)	GH	30	133	75–179	.224
	Other	28	136	99–198	
VII (%)	GH	30	107.5	59–149	.882
	Other	28	110.5	43–185	
VIII (%)	GH	30	68.5	34–116	.143
	Other	28	75.7	31–225	
IX (%)	GH	30	97.8	48–157	.565
	Other	28	102.5	43–159	
X (%)	GH	30	99	73–132	<.01[b]
	Other	28	112	86–143	
vWF (%)	GH	30	85.6	54–122	.04
	Other	28	103.5	44–172	
Antithrombin (%)	GH	30	123	103–145	.975
	Other	28	120.3	103–146	
Fibrinogen (mg/dL)	GH	30	251	161–512	<.01[b]
	Other	28	348	227–577	

[a]Units of measurement are recorded as a % of a canine standard that had an assigned value of 100% for all coagulation factors, antithrombin, and vWF : Ag and mg/dL for fibrinogen.

[b]GH types have significantly lower levels of coagulation factors/hemostatic proteins.

Discussion

Previously published medical and veterinary research indicates that certain coagulation factor and hemostatic proteins—factor VIII and vWF—are unstable proteins and they decrease within plasma over time during certain storage conditions.[7,16,17] The results of the current study suggest that a longer period of time between collection of whole blood units, and processing of these units does not adversely affect CF&HPC in end-product frozen plasma. The median coagulation factor activities for all units were above 50%, and well above the minimum values considered necessary to support surgical hemostasis in human transfusion practice.[18,19]

There was a wide interindividual variation in factor VIII content among dogs compared with other factors/proteins; however, the values within each group did not show a decreasing factor level as processing delay increased. It would be expected that factor activity would deteriorate to a degree with prolonged whole blood storage preprocessing, as has been seen in similar human studies.[16,17] In contrast, the study demonstrated a significantly higher level of factor VIII in the frozen plasma prepared after 24 hours compared with the frozen plasma prepared after 8 and 12 hours. The increased levels of factor VIII and several other factors in the frozen plasma prepared after 24 hours could reflect generation of thrombin and conversion of procofactors to their functional form, perhaps

mediated by leukocytes, platelets, and elaborated cytokines or autoactivation of the contact pathway factors during the preprocessing storage time as whole blood units.[5,8,20,21]

Leukoreduction—which is standardly performed within the NHS BT at processing (<24 hours from venepuncture)[2] could be a procedure to consider as none of the units in this study were leukoreduced. Leukoreduction might reduce ex vivo activation of coagulation, but as it occurs at processing, its effect on this could be limited and as a practical consideration it would increase unit production cost. This is an area that the authors feel further studies at blood bank level could be useful. Clinical studies using end product are also warranted.

The factor VIII variability leads to concerns about uniformity and standard potency of cryoprecipitate when being used to treat hemophilia A. Cryoprecipitate units are currently prepared from single-donor plasma units. Results of this study suggest that alternative methods of production for cryoprecipitate such as pooling of multiple units from individual donors or plasma fractionation need to be considered in canine blood banking to produce a more reliable product for such specific indications. Further work is required to address this variability in factor VIII.

Greyhounds are overrepresented compared with their proportion of the dog population as canine blood donors internationally.[9] We found generally lower CF&HPC in plasma units collected from Greyhound types versus other breeds, and were statistically significant for factor X activity, VWF : Ag, and fibrinogen.[10,11] A possible explanation of this finding is that the high hematocrit of Greyhound types results in an altered ratio and relative excess of citrate in their plasma units. When the plasma is combined with substrate deficient plasma in the modified APTT and PT factor assays, the excess citrate binds to free-ionized calcium and artifactually delays clotting time.[22] In addition to this preanalytic variable influencing clotting time tests, somewhat lower VWF : Ag in the Greyhound breed has been reported in a previous study.[10]

Limitations of this study were that we did not monitor the same unit over time; rather, individual units from different dogs were compared for the 3 different processing groups. The number of samples used in this study was also limited; a larger study number would have enabled more conclusions to be drawn from any significant results.

Conclusion

This study indicated that processing of whole blood to FFP within 24 hours of collection is a suitable alternative to production of FFP within 8 hours of collection. Greyhound types had significantly lower concentrations of vWF, factor X, and fibrinogen when compared with other breeds, but are still deemed suitable for use as FFP donors.

Footnotes

[a] Macopharma quadruple cpd/sag-m, Macropharma, Middlesex, UK
[b] Sorvall RC-3C with rotor H6000A, Thermo Scientific, Hampshire, UK
[c] Sanyo Ultra Low Temperature Freezer MDF-U32V-80, Loughborough Leics, UK
[d] Diagnostica Stago, Pasippany, NJ
[e] Thromboplastin LI; Helena Diagnostics, Beaumont, TX
[i] SPSS v16, Chicago, IL

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