Research Article

The effects of prestorage leukoreduction and storage duration on the in vitro quality of canine packed red blood cells

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Abstract: The effects of prestorage leukoreduction and storage duration on the in vitro quality parameters of canine packed red blood cells (pRBCs) stored in saline–adenine–glucose–mannitol (SAGM) were investigated. Hematological, biochemical, and morphological changes in filtered and unfiltered pRBCs were determined each week during 42 days of storage. All of the parameters changed significantly during storage. The effects of leukoreduction on leukocyte and erythrocyte count, packed cell volume, hemoglobin content, and erythrocyte morphology were significant. While supernatant K increased during storage, glucose, Cl, and 2,3-diphosphoglycerate (2,3-DPG) concentrations decreased. Filtered pRBCs had higher 2,3-DPG levels throughout storage than their unfiltered counterparts. It was concluded that the leukoreduction filter significantly decreases the number of leukocytes and that canine pRBCs can be stored in SAGM for 42 days. Furthermore, leukoreduction may be a consideration with the intention of improving oxygen delivery, since the filtered pRBCs had a higher 2,3-DPG concentration.

Key words: Dog, erythrocyte, blood storage, SAGM, leukocyte filtration

Introduction

The primary purpose of transfusion therapy is to replenish the specific cellular or noncellular blood component that is diminished. Due to improvements in component preparation technology, the use of blood products instead of whole blood has become a common therapeutic practice (1,2). Red blood cell (RBC) concentrates can be stored in commercially available multiple bag systems containing various additive solutions for up to 42 days (3–5). However, the structure and metabolism of the RBCs differ significantly among species (6,7). Therefore, the storage time of packed RBC (pRBC) units in the same solution varies between species (2,4,8).

Despite the use of additive solutions, during storage erythrocytes undergo some complex structural, functional, and metabolic alterations, which are called storage lesions (3–5,9). These changes damage the cells irreversibly and ultimately reduce the function and survival of RBCs following transfusion (10,11). White blood cells (WBCs) and their byproducts present in the pRBC units also influence the quality of blood components (9,10,12,13). In humans, WBCs present in RBC units are associated with several posttransfusion reactions and the removal of WBCs from blood components may reduce the incidence of such reactions (11–14). Furthermore, it has been indicated that the metabolic parameters of the leukoreduced

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(LR) pRBCs are preserved better and they have longer shelf lives (10,12,15). Leukoreduction might also be beneficial in canine transfusions. However, the efficiency of currently available commercial leukocyte filters designed for use in human blood has not been investigated extensively in canine blood. In the current study, we aimed to investigate the effects of prestorage leukocyte filtration and storage duration on some hematological, biochemical, and morphological parameters of canine pRBCs stored in saline–adenine–glucose–mannitol (SAGM) solution for 42 days.

Materials and methods

Fifteen clinically healthy male Kangal dogs (>30 kg) were used in the study. All of the procedures were approved by the Animal Care and Ethics Committee of İstanbul University's Faculty of Veterinary Medicine.

From each dog, 1 unit (450 mL/unit) of whole blood was collected into a triple-bag blood collection system (JMS CPD-SAGM Triple Blood Bag). The blood was initially collected into a primary bag that contained 63 mL of citrate–phosphate–dextrose as an anticoagulant. After centrifugation (4200 × g, 15 min, 0 °C), the plasma was extracted to the empty satellite bag using a manual plasma extractor, and the additive solution SAGM in the second satellite bag was transferred to the primary bag that contained the pRBCs. After being resuspended in 100 mL of SAGM, half of the pRBCs were filtered with a leukoreduction filter (Imugard III RC-4P) (LR group), and the remaining half was left unfiltered (nLR group) in the primary bag. The filtered and unfiltered pRBC units were stored at 4 °C in a blood bank refrigerator for 42 days. Samples were taken aseptically on days 0, 7, 14, 21, 28, 35, and 42 of storage for hematological and biochemical analysis.

The WBC count in the nLR units, RBC count, packed cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), and RBC distribution width (RDW) were determined using an automated hematology analyzer. The WBC counts of the filtered units (LR group) were performed manually using a Nageotte counting chamber (12,16). RBC morphology was assessed on May–Grünwald/ Giemsa-stained slides by light microscopy (17). A discocyte obtained a score of 0, and echinocytes were graded from +1 to +3 as follows: echinocyte I (+1), irregularly contoured discocyte with up to 5 protrusions; echinocyte II (+2), flat cell with multiple spicules; and echinocyte III (+3), ovoid or spherical erythrocyte with multiple spicules. For each sample, 200 RBCs were scored and the morphological index (MI) was calculated as Σ scores / 200 (17). Supernatant glucose, Na, K, and Cl concentrations were measured using an automated analyzer. The concentration of 2,3-diphosphoglycerate (2,3-DPG) was determined spectrophotometrically by enzymatic assay (Roche Diagnostics, Cat. No. 10 148 334 001) on days 0, 14, 28, and 42.

Repeated measurement of ANOVA in SPSS 10.0 was used to analyze the data for hematological and biochemical parameters. The model included sampling day as a within-subject effect and group as a between-subject effect, and also sampling day × group interaction. Significance control was assessed using the least significant difference procedure. To determine the effect of the group on hematological and biochemical parameters for the specific sampling day, paired samples t-tests were also performed.

Results

All of the hematological parameters, except for the RBC number in the nLR group, changed significantly during storage (Table 1). The nLR group had a significantly higher WBC count than the LR group throughout the storage period (P < 0.001). The LR group had a higher RBC and Hb level than the nLR group. Mean RDW values in the nLR group were higher than those of the LR group after 21 days of storage. During storage, the MI increased from 0.12 ± 0.01 to 0.63 ± 0.03 in the nLR group and from 0.16 ± 0.03 to 0.43 ± 0.02 in the LR group (P < 0.001).

Glucose concentration decreased significantly throughout storage in both groups (P < 0.001); however, the difference between the groups was not significant on any sampling day of the study (Table 2). K increased significantly during storage in both groups (P < 0.001). Cl showed similar changes in both groups and it decreased significantly during storage (P < 0.001). The concentration of 2,3-DPG

Table 1. Hematological parameters of the filtered (LR) and unfiltered (nLR) canine packed RBC units stored in SAGM for 42 days.

Trait	Group (G)	0	7	14	21	28	35	42	SEM	Sig.^{y}	G	D	G × D
WBC (× 10³/µL)	nLR	15.93^{ab}	15.87^{ab}	15.47^{ab}	15.29^{b}	15.09^{b}	15.05^{b}	16.20^{a}	0.841	*			
	LR	0.02^{d}	0.02^{d}	0.06^{c}	0.09^{b}	0.14^{a}	0.14^{a}	0.13^{ab}	0.009	***	***	*	*
	Sig.^{x}	***	***	***	***	***	***	***					
RBC (× 10⁶/µL)	nLR	5.86	5.87	6.21	6.06	5.79	5.92	5.78	0.274	NS			
	LR	6.37^{c}	6.98^{b}	8.24^{a}	7.96^{a}	7.97^{a}	8.54^{a}	8.22^{a}	0.143	***	***	***	***
	Sig.^{x}	NS	**	***	***	***	***	***					
PCV (%)	nLR	38.97^{c}	39.09^{c}	40.17^{bc}	41.79^{a}	40.33^{ab}	40.47^{ab}	39.24^{bc}	1.499	*			
	LR	50.26^{a}	43.91^{cd}	43.89^{d}	45.56^{bc}	44.45^{c}	45.61^{b}	45.70^{bc}	0.747	***	**	***	***
	Sig.^{x}	***	*	NS	NS	NS	*	**					
Hb (g/dL)	nLR	13.05^{c}	13.56^{b}	13.88^{ab}	13.99^{a}	13.59^{b}	13.48^{bc}	13.29^{bc}	0.567	**			
	LR	15.94^{c}	17.57^{ab}	17.62^{c}	18.47^{ab}	18.11^{b}	18.32^{ab}	18.63^{a}	0.514	***	***	***	***
	Sig.^{x}	**	***	**	***	***	***	***					
MCV (fL)	nLR	66.67^{d}	66.73^{cd}	67.40^{cd}	69.20^{ab}	69.93^{a}	68.53^{b}	67.47^{bc}	0.732	***			
	LR	66.53^{d}	66.53^{d}	67.13^{c}	69.00^{a}	69.20^{a}	68.00^{b}	66.27^{d}	0.692	***	NS	***	NS
	Sig.^{x}	NS	NS	NS	NS	*	NS	**					
RDW (%)	nLR	14.54^{bc}	14.61^{cd}	14.86^{c}	14.98^{bc}	14.83^{cd}	15.44^{b}	16.95^{a}	0.330	***			
	LR	14.34^{bc}	14.90^{a}	14.85^{a}	14.44^{b}	14.07^{cd}	14.01^{d}	13.78^{d}	0.266	***	NS	***	***
	Sig.^{x}	NS	NS	NS	**	**	***	***					
MI	nLR	0.12^{e}	0.23^{c}	0.28^{d}	0.40^{c}	0.43^{c}	0.50^{b}	0.63^{a}	0.011	***			
	LR	0.16^{d}	0.19^{d}	0.09^{c}	0.09^{c}	0.35^{b}	0.40^{a}	0.43^{a}	0.011	***	***	***	***
	Sig.x	NS	NS	***	***	*	**	***					

NS = not significant (P > 0.05), *: P < 0.05, **: P < 0.01, and ***: P < 0.001.

^{x}: Significance level of the differences between groups for the same storage time according to paired samples t-test statistics.

^{x}: Significance level of the differences between storage times for the same group according to repeated measurements of ANOVA statistics.

^{y}: Differences between the means of sampling times carrying various letters in the same row are significant.

$^{a, b, c, d, e}$: Differences between the means of sampling times carrying various letters in the same row are significant.

WBC, white blood cell count; RBC, red blood cell count; PCV, packed cell volume; Hb, hemoglobin; MCV, mean corpuscular hemoglobin; RDW, RBC distribution width; MI, RBC morphological index; SEM, standard error of the mean.

Table 2. Biochemical parameters of the filtered (LR) and unfiltered (nLR) canine packed RBC units stored in SAGM for 42 days.

Trait	Group (G)	Day (D)							SEM	Sig. ʸ	Significance of main effects		
		0	7	14	21	28	35	42			G	D	G × D
Glucose (mg/dL)	nLR	706.07ᵃ	617.80ᵇ	585.47ᵇ	511.73ᶜᵈ	512.47ᶜ	467.13ᵈ	429.80ᵉ	22.839	***	NS	***	NS
	LR	727.93ᵃ	615.33ᵇ	587.13ᵇ	542.27ᶜ	538.13ᶜ	491.60ᵈ	451.47ᵉ	20.257	***			
	Sig. ˣ	NS	NS	NS	NS	NS	NS	NS					
Na (mmol/L)	nLR	169.87ᵇ	167.80ᵇᶜ	170.73ᵃᵇ	165.93ᶜ	172.53ᵃ	165.73ᶜ	167.33ᶜ	1.115	**	***	***	NS
	LR	165.87ᵃᵇ	155.07ᵈ	165.73ᵃᵇ	160.47ᶜᵈ	168.67ᵃ	164.00ᵇᶜ	164.93ᵃᵇᶜ	0.837	***			
	Sig. ˣ	**	**	*	***	**	NS	NS					
K (mmol/L)	nLR	1.78ᶠ	3.04ᵉ	3.21ᵈ	4.01ᶜ	4.45ᵇ	4.68ᵃ	4.73ᵃ	0.217	***	NS	***	***
	LR	1.59ᵈ	3.67ᶜ	3.77ᶜ	4.17ᵇ	4.42ᵃᵇ	4.47ᵃ	4.43ᵃ	0.158	***			
	Sig. ˣ	*	*	*	NS	NS	NS	NS					
Cl (mmol/L)	nLR	123.67ᵃ	116.67ᵇ	113.40ᶜ	113.87ᵇᶜ	111.33ᵈ	109.87ᵉ	111.47ᵈ	2.059	***	NS	***	NS
	LR	127.53ᵃ	119.80ᵇ	116.07ᶜ	116.00ᶜ	113.87ᵈ	112.07ᵉ	113.00ᵈᵉ	1.218	***			
	Sig. ˣ	*	NS	NS	NS	NS	NS	NS					
2,3-DPG (g/L)	nLR	0.52ᵃ		0.40ᵃ		0.18ᵇ		0.10ᶜ	0.038	***	***	***	***
	LR	0.90ᵃ		0.79ᵃ		0.41ᵇ		0.22ᶜ	0.030	***			
	Sig. ˣ	***		***		***		***					

NS = not significant (P > 0.05), *: P < 0.05, **: P < 0.01, and ***: P < 0.001.

ˣ: Significance level of the differences between groups for the same storage time according to paired samples t-test statistics.

ʸ: Significance level of the differences between storage times for the same group according to repeated measurements of ANOVA statistics.

ᵃ, ᵇ, ᶜ, ᵈ, ᵉ, ᶠ: Differences between the means of sampling times carrying various letters in the same row are significant.

Na, supernatant sodium; K, supernatant potassium; Cl, supernatant chloride; 2,3-DPG, 2,3-diphosphoglycerate concentration; SEM: standard error of the mean.

decreased significantly during storage in both groups ($P < 0.001$), and it was significantly lower in the nLR group than in the LR group throughout the storage.

Discussion

According to the standards in human blood banking, leukocyte content below (5×10^6)/unit is required if a pRBC unit is to be considered leukoreduced (18). In the current study, the leukoreduction filter caused a significant decrease in the WBC number of the filtered units. However, the WBC number in the LR group was still above the standards. This might result from noncooling of the pRBC units before filtration. Supporting this, Brownlee et al. (16) found a 99.9% reduction in WBCs after filtration of cooled blood, whereas only a reduction of 88.9% could be achieved after filtration of noncooled blood.

The alterations observed in the RBC count, Hb level, and PCV were comparable to those reported for canine pRBCs stored in other additive solutions (8). However, the PCV values determined in the current study were lower than those reported for canine pRBCs stored in Nutricel (2) or Adsol (1). This might have resulted from the decreased filling of blood collection bags (19). Furthermore, although a PCV of 55%–65% is the aim for pRBCs stored in additive solutions (4,5,9), the PCV of a pRBC unit may vary depending on the PCV of the donor, diluting effects of the additive solutions, and the amount of residual plasma volume in the bag (5,9,20). The RBC count, PCV, and Hb level were significantly higher in the LR units than in the nLR units (Table 1). This might be the result of sedimentation that occurred during the filtration and preparation of the pRBC units (1,8). During filtration, the sedimentation that occurred in the primary bag, which contained the nLR RBCs, caused the transition of more RBCs to the empty satellite bag, which contained the LR pRBC unit. Similar to the current results, an increase in MCV has also been reported for stored canine (8) and human (17) RBCs. The increase in MCV might result from the insufficiency of the adenosine-5'-triphosphate (ATP)-dependent Na–K pumps of the RBC membrane, which cannot work properly under cold storage conditions (17). The type of the anticoagulant used for storage might also affect the

MCV (19). The higher MCV value determined on day 42 in the nLR group might have resulted from the products of leukocytes, which are known to affect erythrocyte membrane properties (17). The higher RDW values observed in the nLR units following 21 days of storage might be an indicator of deformation and anisocytosis in the erythrocytes (17).

Membranous changes that occur during storage decrease the elasticity of the erythrocytes and affect their rheological properties (15,17). Erythrocytes must maintain flexibility and should pass quickly through the capillaries in order to supply an adequate level of oxygen to the tissues (11,21). An increase in the MI could impair this function, as echinocytes are more rigid than discocytes and cause an increase in blood viscosity (9,15,17). A gradual echinocytic shape transformation, which is evident from the increased MI, was observed for canine pRBCs in the current study. The nonsignificant difference observed in the MI between the groups on day 0 indicates that the filtration process did not affect the normal discocytic shape of canine RBCs (15,17). However, significantly higher MI values determined in the nLR group after 14 days of storage suggest that the presence of leukocytes in canine pRBCs contribute to echinocytic shape transformation.

The gradual decrease of glucose concentration (39%) observed in the current study was similar to those reported for canine (8,22), equine (19), and human (12,20) RBCs stored in various additive solutions. However, the levels of decrease in the glucose concentration for canine pRBCs stored in CPDA-1 reported by Price et al. (8; 85%) and Wardrop et al. (22; 93%) were higher than the current results. The lower glucose consumption determined in the current study might have resulted from different formulation of additive solutions or from different responses of the RBCs to the storage media (22).

The most dramatic change that occurs in electrolytes during storage is the increase of supernatant K (3,23). Significant alterations detected in the current study for supernatant K, Na, and Cl concentrations are parallel to those reported for stored canine (8,22), equine (7,19), rat (6), and human (6,20) RBCs. Most of the changes identified in the electrolytes are probably due to storage-induced ATP depletion,

which affects the membrane transport mechanisms (8,22,23). During storage, while the concentration of glucose decreases, the amounts of lactic and pyruvic acids increase and cause a decline in pH, which in turn causes the depletion of erythrocyte ATP and 2,3-DPG (5,9,11,19,23). As the ATP concentrations decline, the Na/K membrane pumps start to fail, and intracellular K leaks into the storage media (7,19). The cold storage conditions, which paralyze and reduce the activity of the ion pumps, also contribute to the leakage of K (3,5,7,14). The progressive rise of K during storage determined in this study is similar to those reported for canine pRBCs stored in Adsol and Nutricel (22), but lower than those reported for canine RBCs stored in CPDA-1 (8,22). This difference might have resulted from the presence of plasma, which contains much more K and Na compared to additive solutions, in RBCs stored in CPDA-1 (22). Although the effect of day was significant on both Na and Cl concentrations, the effect of group was only significant on the Na level (Table 2). Significant time-related changes in supernatant Na concentrations have also been reported for canine pRBCs stored in CPDA-1, Adsol, and Nutricel (8,22). However, Mudge et al. (19) did not find any significant change in the concentrations of serum Na and Cl during the storage of equine pRBCs.

2,3-DPG, which is routinely used to assess the in vitro quality of stored RBCs, showed a typical pattern of loss during storage in both groups (Table 2). The rate of decline in the 2,3-DPG concentration varies among species. In human RBCs, 2,3-DPG decreases to near a nondetectable level within 2 weeks of storage (6,24), whereas in rat RBCs, it decreases by more than 90% on day 8 of storage (6). On the other hand, equine RBCs had half of their original 2,3-DPG reserves after 35 days of storage (7). In the current study, canine pRBCs had 88% and 77% of their initial 2,3-DPG concentrations by day 14 in the LR and nLR groups, respectively. Furthermore, the declines in 2,3-DPG concentrations found in the current study were comparable to those seen after the storage of canine pRBCs in other additive solutions (1,2,8). Wardrop et al. (1) reported that canine pRBCs stored in Adsol had 87% of their initial 2,3-DPG concentration by day 10 of storage, and the decrease in 2,3-DPG

level was significant after 20 days of storage. Canine pRBCs stored in Nutricel had approximately more than half of their initial 2,3-DPG reserves after 35 days of storage (2).

2,3-DPG has great importance for oxygen transport after transfusion because it modulates oxygen delivery to the tissues (7,19). Therefore, decreased 2,3-DPG concentrations may reduce oxygen delivery to peripheral tissues, which in turn influences the patient's morbidity and mortality (10,11,19,21). The decrease in 2,3-DPG is restored within 24–72 h after transfusion in humans (11,23). However, the degree of reversibility of storage-induced 2,3-DPG depletion in dogs is unknown (1), and it may be irreversible (8). Furthermore, Duhm (25) found significant differences in the ability of RBCs to produce DPG after incubation with a PIPA solution between species, and he reported that while large amounts of 2,3-DPG are produced in the erythrocytes of mouse, rabbit, human, and pig, only small amounts of 2,3-DPG are formed in RBCs from rat, dog, and guinea pig. Therefore, the higher maintenance of 2,3-DPG level in stored canine RBCs may be an advantage over human RBCs. In the current study, the LR units had higher 2,3-DPG levels throughout the storage period than their nLR counterparts. Therefore, in order to improve oxygen delivery, leukocyte filtration may be a consideration when transfusing stored blood during anesthesia or to patients with acute severe anemia (8,19).

It is concluded that the leukoreduction filter used in the current study effectively removes leukocytes from pRBC units, and canine RBCs can be stored in SAGM for 42 days before transfusion. Furthermore, because of the higher 2,3-DPG level determined in the filtered units, prestorage leukoreduction may be a consideration when stored blood is transfused with the purpose of improving oxygen delivery.

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