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Effects of cryopreservation and leukodepletion on morphological index of red blood cells in dogs

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Short Communication

Effects of cryopreservation and leukodepletion on morphological index of red blood cells in dogs

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Abstract

Preservation of blood components for transfusion is a long-standing phenomenon. However, studies of cryopreservation of leukodepleted canine erythrocytes are quite limited and no studies are available with respect to morphological evaluation of erythrocytes during the cryopreservation process. The aim of this study was to determine morphological changes caused by cryopreservation, storage and leukocyte filtration. Healthy adult dogs meeting the criteria for blood transfusion were used in this study. Packed red blood cells (pRBCs) were obtained from each dog. The samples were divided into two groups. Leukocyte filtration was performed on one group of pRBCs. The other group received no filtration. The samples of each group were then allocated into three sub-groups according to storage period and subjected to glycerolization (40% w/v Glycerol) prior to cryopreservation except for day 0 group. Glycerolized samples were stored at -80°C for four and six months. Blood smears were obtained from the samples of day 0 and those thawed and deglycerolized at the end of the storage period. The smears were morphologically evaluated by light microscopy, and Morphological Index (MI) was calculated. MI values of the non-leukodepleted (nLD) and leukodepleted groups (LD) increased from 0.083 and 0.119 to 0.238 and 0.273, respectively. In conclusion, leukocyte filtration did not have impact on the MI values of leukofiltrated and glycerolized pRBC samples but the cryopreservation and storage period did.

Keywords: cryopreservation, dog, erythrocyte, leukodepletion, morphological index

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Introduction

Preservation of blood for transfusion has been a significant progress in saving lives. Degradation of blood components and storage of blood in plastic bags in liquid form are long-standing processes (Pallota et al., 2012).

Cryoprotectant agents have been developed to prevent possible cellular damages, since frozen storage of mammary cells may be a harmful process due to the occurrence of ice crystals during freezing and thawing steps. In addition, cryopreservation may trigger certain physiological changes such as alterations in intra- and extracellular material concentrations (Pallota et al., 2012; Wagner et al., 2000; Chaudhari, 2009). Glycerol is one of the most widely used agents in cryobiology as it is a non-toxic substance at high concentrations and can easily diffuse into cell at 37°C (Pallota et al., 2012). Two different glycerolization protocols are applied for cryopreservation of RBCs depending on glycerol concentration (15-20% or 40% w/v), speed of freezing (slow or rapid) or storage temperature (-196°C or -80°C) (Pallota et al., 2012; Wagner et al., 2000). RBC deformity is a major determinant of blood viscosity at high cut-off centrifuging speed (Sollberger et al., 2002).

Functional and structural changes may occur in erythrocytes during storage. These changes were reported to contribute to certain pathological changes such as the transition of erythrocytes into spheroechinocytes during storage (Berezina et al., 2002; Holme, 2005). The presence of leukocytes and enzymes released from leukocytes and cytotoxic mediators in RBC units during storage period all affect the quality of blood components (Chin-Yee et al., 1997; AuBuchon et al., 1997; Walter et al., 2000; Hess and Greenwalt, 2002; Zehnder et al., 2008; Hess, 2010). Marked morphological changes such as outward budding of microvesicles or spicule formations (generally named echinocytes) have been demonstrated on the erythrocyte membrane particularly during storage. Furthermore, storage deteriorates rheological properties of blood components stored in the form of whole blood (Walter et al., 2000). In contrast, metabolic parameters are well maintained in leukodepleted packed red blood cells (LD-pRBCs) and shelf life is extended (Chin-Yee et al., 1997; AuBuchon et al., 1997; Walter et al., 2000). Irreversible changes and functional losses seen in RBCs during storage hamper their survival after transfusion (Chin-Yee et al., 1997; Vandromme et al., 2009; Hess and Greenwalt, 2002) and even hinder the whole transfusion process (Wardrop, 1995). However, leukocyte depletion was not found to exhibit a significant effect on quality parameters of canine pRBCs that were cryopreserved by glycerol (Bala et al., 2016).

Therefore, the objective of the present study was to determine whether cryopreservation period and leukocyte depletion have impact on the MI parameter in canine pRBC products.

Materials and Methods

Fourteen healthy adult dogs meeting the criteria for blood transfusion were used in the study. The experimental procedure was approved by the Local Ethics Committee for Animal Experiments of Istanbul University, Faculty of Veterinary Medicine (Approval no. 2009/122). Whole blood (450 ± 45 ml) was collected from each donor into CPDA-1 bags. The samples were then centrifuged in a refrigerated centrifuge (4200 rpm) at 22°C for 5 min and pRBCs were obtained. Then, the samples were divided into 2 equal groups. The first group of pRBCs was passed through leukocyte filters (Imugard III RC-4P) and the second group was left intact. Leukocyte count of the filtrated samples was determined by the Neagotte hemocytometer and estimated at log level (Bontadini et al., 2002). The filtrated and non-filtrated samples were rinsed 3 times in 0.9% NaCl (Brecher, 2005). Next, the samples were allocated into 3 sub-groups according to storage periods: 0 day, 4 months and 6 months. Samples to be frozen were treated with 40% w/n glycerol (Glycerolyte 57) and then stored at -80°C for 4 and 6 months. At the end of the storage period, the pRBC and LD-pRBC samples were thawed at 36-38°C and then washed in 12%, 1.6% and 0.9% NaCl + 0.2% dextrose solutions, respectively (Wagner et al., 2000). Blood smears were obtained from the samples of day 0 and those thawed and deglycerolized (Valeri et al., 2000; Kim et al., 2004). Afterwards, the glasses were stained with May-Grünwald/ Giemsa Stain and morphologically evaluated by light microscopy. The scoring system was based on the occurrence of discocytes and echinocytes: Discocyte, 0; Echinocyte 1 (irregularly shaped discocyte with maximum of 5 spicules), +1; Echinocyte 2 (well-shaped discocyte with more than one spicule), +2; Echinocyte 3 (ovoid or spherical erythrocyte with more than one spicule), +3. Two hundred erythrocytes were scored for each sample and Morphological Index (MI) was calculated (MI = Σ scores / 200) (Sollberger et al., 2002).

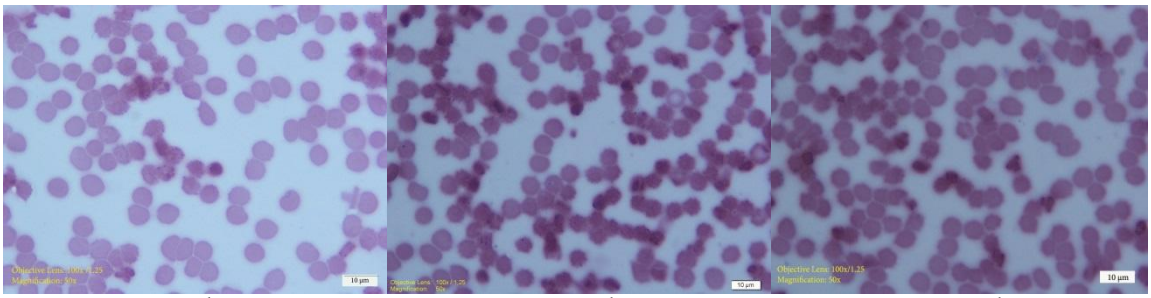
Statistical analysis was performed using the SPSS-software package (ver. 11.5.2.1, SPSS Inc., Chicago, IL, USA). Since our data were not normally distributed, the non-parametric Kruskal-Wallis test was used for statistical analysis. The Mann-Whitney U test with Bonferroni correction was used for pairwise comparisons (post-hoc tests).

Results and Discussion

Leukocyte count of the LD group was determined at 2.6 $\log_{10}$ level. MI values of the non-leukodepleted (nLD) and leukodepleted groups (LD) increased from 0.083 and 0.119 to 0.238 and 0.273, respectively. No significant difference was noted between the nLD and LD groups in terms of MI values of the freezing process. The filtration prior to freezing did not alter the discoid form of the erythrocytes (Figure 1). However, the freezing and storage period affected the morphological properties of erythrocytes in the samples of LD and in those of nLD+LD, respectively (Table 1).

Micromorphological changes occurred on the frozen erythrocytes ranging from characteristic biconcave discoid form to echinocyte and then to spherocyte forms. Cells eventually lose their borders by developing echinocytic spicules and microvesicle budding on their membranes (Walter et al., 2000; Hess

and Greenwalt, 2002). Smaller vesicles cause greater energy loss on the surface area of the erythrocytes. And finally, storage process results in hemolysis. Furthermore, membrane damage is considered to be irreversible at this stage (Hess and Greenwalt, 2002; Hess 2010). One of the most efficient ways to prevent membrane damage is to store the products in polyvinyl chloride (PVC) bags (Hess and Greenwalt, 2002) as was done in the study.



0 day 4 months 6 months

Figure 1 Cell changes in storage process

Table 1 Effects of cryopreservation and leukodepletion on morphological index of red blood cells in dogs

Storage period	Group (G)			
	nLD RBC (n=12)		LD RBC (n=12)	
	Means	SD	Means	SD
0 day	0.083[a]	0.013	0.119[a]	0.016
4 months	0.137[a]	0.017	0.197[a]	0.021
6 months	0.238[b]	0.022	0.273[b]	0.015
Significance[A] (P-value)	**<0.005**		**<0.005**	

[A] Repeated measures analysis of variance for each group indicates significance.
[a, b] There is a significant difference between the means indicated with different letters in the same column.
No difference was noted between the leukodepleted (LD) and non-leukodepleted (nLD) groups on 0 day or at 4 and 6 months. Differences indicated by the different superscript letters in each group according to the different freezing periods were statistically significant at $p<0.05$ level.

Increases in the MI values of the LD and nLD groups were found to be statistically insignificant (Table 1). That leukodepletion did not have impact on the MI parameter and the normal biconcave form of the erythrocytes, similar to the findings of Walter et al. (2000), who studied human erythrocytes. The findings were also compatible with those of Sollberger et al. (2002), who compared the effects of leukocyte depletion and storage period at low temperature on human erythrocytes. In addition, Ekiz et al. (2012) reported that filtration under normal storage conditions did not alter the usual discocytic form of canine RBCs and MI values varied insignificantly among groups. Similar to our study, Notomi et al. (2016) indicated that filtration did not lead to significant changes in the MI values but the MI values increased significantly after storage.

Freezing did not significantly affect the erythrocytes in the nLD group, whereas the increase in the MI value of the LD group was statistically significant (Table 1). Glycerolization was previously reported to cause significant alterations in the cellular membranes of fresh and frozen human RBCs (Pallota et al., 2012). However, in our study, it is suspected that the changes in the MI value of the LD group might result from the pre-treatment procedures. Similarly, Lecak et al. (2004) reported that cellular damage might occur during the pre-treatment stage due to a mechanical injury.

Lecak et al. (2004) investigated the effect of freezing period and detected certain morphological changes and cellular damages on RBCs that were stored at -80°C for 10 years, yet these changes were negligible. In our study, the storage period caused an increase in the MI value in both groups ($p < 0.05$). Several researchers established a link between the level of ATP loss during storage and the morphological changes that occurred during this period (Chin-Yee and d'Almedia, 1997; Zubair, 2010). Kim et al. (2004) indicated that occurrence of osmotic stress during glycerolization and deglycerolization might cause morphological changes in canine erythrocytes. In another study, osmotic fragility of human and canine erythrocytes was compared, and it was indicated that human RBCs were less fragile than those of dogs (Matsuzava and Ikarashi, 1979). Based on these findings, our study suggests that the application of glycerol during freezing of canine erythrocytes along with the storage period led to osmotic stress and concurrent ATP loss, which resulted in the increase in MI value.

Conclusion

Our findings imply that leucodepletion and freezing through glycerolization performed on canine pRBCs had no impact on erythrocyte morphology; however, osmotic stress occurring during cryopreservation rendered the cells more fragile.

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References

AuBuchon JP, Elfath MD, Popovsky MA, Stromberg RR, Pickard C, Herschel L, Whitley P, McNeil D, Arnold N, O'Connor JL 1997. Evaluation of a new prestorage leukoreduction filter for red blood cell units. Vox Sang. 72(2): 101-106.

Bala DA, Eraslan E, Akyazi I, Ekiz EE, Ozcan M, Cotelioglu U and Arslan M 2016. Freezing and storage of leukodepleted erythrocyte suspensions. Vet Med Czech. 61(8): 443-448.

Berezina TL, Zaets SB, Morgan C, Spillert CR, Kamiyama M, Spolarics Z, Deitch EA and Machiedo GW 2002. Influence of Storage on Red Blood Cell Rheological Properties. J Surg Res. 102(1): 6-12.

Bontadini A, Tazzari PL, Manfroi S, Tassi C and Conte B 2002. Apoptosis in leukoreduced packed red blood cells. Vox Sang. 83: 35-41.

Brecher ME 2005. AABB Technical Manual. In: Blood collection, storage and component preparation (15th ed): Bethesda, Maryland, p. 799-818.

Chaudhari SCC 2009. Frozen Red Blood Cells in Transfusion. Med J Armed Forces India. 65(1): 55-58.

Chin-Yee I, Arya N, d'Almeida MS 1997. The red cell storage lesion and its implication for transfusion. Transfus Sci. 18(3): 447-458.

Ekiz EE, Arslan M, Akyazi I, Eraslan UE, Gültekin GI, Özcan M 2012. The effects of prestorage leukoreduction and storage duration on the in vitro quality of canine packed red blood cells. Turk J Vet Anim Sci. 36(6):711-717.

Hess JR 2010. Red cell changes during storage. Transfus Apher Sci. 43(1): 51-59.

Hess JR, Greenwalt G 2002. Storage of red blood cells: new approaches. Transfus Med Rev 16(4): 283-295.

Holme S 2005. Current İssues Related to the Quality of Stored RBCs. Transfus Apher Sci. 33(1): 55-61.

Kim H, Tanaka S, Une S, Nakaichi M, Sumida S, Taura Y 2004. A comparative study of the effects of glycerol and hydroxyethyl starch in canine red blood cell cryopreservation. J Vet Med Sci. 66(12): 1543-1547.

Lecak J, Scott K, Young C, Hannon J, Acker JP 2004. Evaluation of red blood cell stored at - 80°C in excess of 10 years. Transfusion. 44(9): 1306-1313.

Matsuzawa T, Ikarashı Y 1979. Haemolysis of various mammalian erythrocytes in sodium chloride, glucose and phosphate-buffer solutions. Lab Anim Sci. 13(4): 329-331.

Notomi MK, Gopegui RR, Escodro PB 2016. Haematologic effects of leukoreduction on canine whole blood post-filtration and post-storage. Comp Clin Path. 25 (1): 145-149.

Pallotta V, D'Amici GM, D'Alessandro A, Rossetti R, Zolla L 2012. Red blood cell processing for cryopreservation: from fresh blood to deglycerolization. Blood Cell Mol Dis. 48: 226-232.

Sollberger T, Walter R, Brand B, Contesse J, Meredith DO, Reinhart WH 2002. Influence of prestorage leucocyte depletion and storage time on rheologic properties of erythrocyte concentrates. Vox Sang. 82(4): 191-197.

Valeri CR, Ragno G, Pivacek LE, Cassidy GP, Srey R, Hansson-Wicher M, Leavy ME 2000. An experiment with glycerol-frozen red blood cell stored at -80°C for up to 37 years. Vox Sang. 79(3): 168-174.

Vandromme MJ, McGwin G, Weinberg JA 2009. Blood transfusion in the critically ill: does storage age matter? Scand. J. Trauma Resusc. Emerg. Med. 17: 35.

Wagner CT, Burnett MB, Livesey SA, Connor J 2000. Red blood cell stabilization reduces the effect of cell density on recovery following cryopreservation. Cryobiology. 41(3): 78-194.

Walter R, Brand B, Mark M, Schnyder L, Stifanic M, Reinhart WH 2000. Effects of leukocyte depletion on rheologic properties of human CPDA-1 blood. Vox Sang. 79(3): 151-155.

Wardrop KJ 1995. Selection of anticoagulant-preservatives for canine and feline blood storage. In: Canine and Feline Transfusion Medicine, Kristensen, A.T. ve Feldman, B.F. (Eds). Vet Clin North Am Small Anim Pract. 25(6): 1263-1276.

Zehnder L, Schulzki T, Goede JS, Hayes J, Reinhart WH 2008. Erythrocyte storage in hypertonic (SAGM) or isotonic (PAGGSM) conservation medium: influence on cell properties. Vox Sang. 95(4): 280-287.

Zubair AC 2010. Clinical impact of blood storage lesions. Am J Hematol. 85(2): 117-122.

บทคัดย่อ

ผลของการเก็บรักษาแบบเย็นและแบบปลอดเม็ดเลือดขาว ต่อดัชนีทางสัณฐานวิทยาของเม็ดเลือดแดงในสุนัข

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การเก็บรักษาส่วนประกอบเลือดสำหรับการถ่ายเลือดเป็นสิ่งสำคัญที่ต้องคำนึงถึง อย่างไรก็ตาม การศึกษาการเก็บรักษาแบบเย็น (cryopreservation) ของเม็ดเลือดแดงสุนัข แบบปลอดเม็ดเลือดขาว (leukodepleted) นั้นมีจำกัด และไม่มีการศึกษาใดที่เกี่ยวข้องกับการประเมินลักษณะทางสัณฐานวิทยาของเม็ด เลือดแดงในระหว่างกระบวนการเก็บรักษาแบบเย็น วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อประเมินการเปลี่ยนแปลงทางสัณฐานวิทยาของเม็ดเลือด ที่เกิดจากการเก็บรักษาแบบเย็น การเก็บ และการกรองเม็ดเลือดขาว โดยศึกษาในสุนัขโตที่มีสุขภาพแข็งแรงและอยู่ในเกณฑ์การถ่ายเลือด โดยเก็บเลือดแบบ เม็ดเลือดแดงอัดแน่น (Packed red blood cells (pRBCs)) จากสุนัข จากนั้นแบ่งตัวอย่างเป็น 2 กลุ่ม กลุ่มที่หนึ่งกรองเม็ดเลือดขาว จาก pRBCs กลุ่มที่สองไม่มีการกรอง จากนั้นตัวอย่างแต่ละกลุ่มจะถูกแบ่งเป็น 3 กลุ่มย่อยตามระยะเวลาในการเก็บรักษา และเติมกลีเซอรอล (40% w / v Glycerol) ก่อนการเก็บรักษาแบบเย็น (ยกเว้นกลุ่มวันที่ 0) การเก็บตัวอย่างแบบเย็นได้เก็บไว้ที่ -80 องศาเซลเซียสเป็นเวลาสี่และหกเดือน จากนั้นทดสอบโดยการตรวจเลือดที่ป้ายบนแผ่นสไลด์ (blood smear) ในวันที่ศูนย์ สี่เดือน และหกเดือนตามลำดับ ผลการประเมินด้วยกล้องจุลทรรศน์แบบใช้แสง พบดัชนีทางสัณฐานวิทยา (MI) ของ non leukodepleted (nLD) และ leukodepleted groups (LD) เพิ่มขึ้นจาก 0.083 และ 0.119 เป็น 0.238 และ 0.273 ตามลำดับ สรุปได้ว่าการกรองเม็ดเลือดขาวไม่มีผลต่อค่า MI ของตัวอย่างเม็ดเลือดแดงที่ผ่านการกรองเม็ดเลือดขาว และเติมกลีเซอรอล แต่มีผลต่อระยะเวลาในการเก็บรักษาแบบเย็นและเวลาในการเก็บรักษา

คำสำคัญ: การเก็บรักษาแบบเย็น สุนัข เม็ดเลือดแดง ปลอดเม็ดเลือดขาว ดัชนีทางสัณฐานวิทยา

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