RESEARCH ARTICLE

Time Dependant Variations in Biochemical Parameters of Canine Packed RBCs Stored in Citrate-Phosphate-Dextrose-Saline-Adenine-Glucose-Mannitol Solution

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Abstract

Metabolites formed during the storage of RBCs determine quality as well as shelf life of blood products. The present study elaborates time-dependent variations of canine-packed red blood cells (pRBCs) stored in citrate-phosphate-dextrose-saline-adenine-glucose-mannitol (CPD-SAGM) solution for 42 days. Whole blood units were collected from volunteer donors into commercially available CPD-SAGM blood bags under aseptic condition. Component separation was carried out to produce pRBCs and fresh plasma. Packed RBCs were stored at 4±2°C in a refrigerator, and samples were taken at two weeks intervals from 0th to 42nd day. Significant variation was recorded in glucose and malondialdehyde (MDA) levels among the biochemical parameters studied during the storage period. With the lengthening of storage time, glucose levels in supernatant decreased while the malondialdehyde levels exhibited a significant increase. Though the variation was insignificant, pH and reduced glutathione (GSH) decreased on storage while supernatant potassium increased from 0thto 42nd day.

Keywords: Biochemical parameters, CPD-SAGM, Malondialdehyde, Packed RBC, Reduced glutathione.

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Introduction

Blood transfusion is an established and life-saving therapeutic protocol in veterinary emergency and critical care medicine. Canine transfusion medicine has advanced from conventional whole blood transfusion to more specific component transfusion. Packed red blood cells (pRBCs) are the most commonly used blood product utilized in companion animal practice. The storage of blood and its components further improved the utility and the effectiveness of this life-saving procedure. Packed RBCs undergo progressive biochemical derangements, morphologic alterations, and oxidative damage on storage. Commonly called 'storage lesions', these alterations adversely affect erythrocyte viability and function. Storage lesions were often attributed to reduced RBC survivability following transfusion (Pavenski *et al*., 2012 and Obrador *et al*., 2015). Additive solutions that preserve the blood product's functional qualities have aided blood storage and its components. Saline-adenine-glucose-mannitol (SAGM) in citrate-phosphate-dextrose (CPD) is a solution available for storing red blood cells. The present study was designed to evaluate the biochemical alterations of canine-packed RBCs stored in CPD-SAGM solution for 42 days.

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Materials and Methods

Preparation of pRBC Units

Commercial triple pack collection systems were used to collect 350 mL whole blood from ten apparently healthy dogs. Each bag contains 49 mL citrate-phosphate-dextrose as anticoagulant and 100 mL saline-adenine-glucose-mannitol as an additive solution. After collection, the triple bag system was placed in the centrifuge cups, and the units were centrifuged at 5,000 × g for 7 minutes, at 4°C using FTBC- 6100R Blood Bank Refrigerated Centrifuge. After centrifugation, plasma was separated into a satellite bag

using a manual plasma expression. The additive solution SAGM in the second satellite bag was transferred to the primary bag that contained the packed RBCs. All these procedures were done inside the horizontal laminar airflow bench to avoid contamination. The packed RBCs suspended in CPD-SAGM solution was stored at 4 ± 2°C in a refrigerator for 42 days for storage studies. The composition of CPD-SAGM solution is given in Table 1.

Estimation of Biochemical Barameters

Aliquots of pRBC units were removed aseptically for analysis on days 0, 14, 28 and 42 of storage to estimate biochemical parameters. Blood pH was estimated with a portable Oakton waterproof pH meter. Potassium was estimated from the supernatant of packed red blood cells using ion-selective electrode technology. Glucose estimation of supernatant of packed RBC was carried out using glucose estimation kit- GenX GLUCOSE-ML (Trinders method). The level of GSH in erythrocyte suspension was determined as per Bain *et al*. (2016). This method was based on a yellow color development when 5,5'- dithiobis-2-nitrobenzoic acid (DTNB) was added to sulphydryl compounds, and the absorbance of the colored complex at 412 nm was quantified using spectrophotometry. Level of MDA was estimated using the method of Okhawa *et al*. (1979) as modified by Al-Azzawieand Alhamdani (2006).

Table 1: Composition of CPD-SAGM solution used for storage of canine pRBCs

Each 49 mL citrate phosphate Dextrose anticoagulant contains	
Citric acid (monohydrate)	0.327 gm
Sodium citrate (dihydrate)	2.630 gm
Sodium dihydrogen phosphate (dihydrate)	0.251 gm
Dextrose (monohydrate)	2.550 gm
Each 100 mL SAGM contains	
Sodium Chloride	0.877 gm
Adenine	0.030 gm
Dextrose (unhydrous)	0.818 gm
Mannitol	0.525 gm

Thiobarbituric acid (TBA) reacts with lipid peroxides and MDA to form a red-colored pigment with maximum absorbance at 532 nm and can be determined by colorimetry.

Statistical Analysis

The statistical analysis of data obtained was done using computer software Statistical Package for Social Sciences (SPSS), version 24.0. Repeated measures ANOVA and pair wise comparison were made using the least significant difference test to compare data.

Results and Discussion

The mean values of biochemical parameters on 0^{th}, 14^{th}, $28^{th,}$ and 42^{nd} day of each blood sample are presented in Table 2.

pH

pH of packed RBCs on day of collection (day 0) was 7.19 ± 3.50. A decline to 6.66 ± 3.72 was recorded on day 42. Mammalian erythrocytes are devoid of mitochondria The sole source of energy for RBCs of these species was ATP produced by glycolysis (Hess, 2010). Lactic acid and pyruvic acid produced during glycolysis resulted in a progressive decline in pH of stored pRBCs. The citric acid present in CPD anticoagulants could have contributed to this pH decline (Rodrigues *et al*., 2020). Increased hydrogen ion activity due to pH fall in stored RBC impairs glycolysis and reduces ATP production (Obrador *et al*., 2015). Adenosine triphosphate is required for RBCs to maintain shape, deformability, membrane elasticity, synthesis of glutathione, purine, and pyrimidine nucleotides. According to Ferreira *et al*. (2018), ATP decline in stored canine RBCs could be delayed by the addition of saline, adenine, glucose, and mannitol in the storage solution. Hess (2006) reported that the storage time of human RBC was determined by time taken to attain a pH of 6.5, below which cells did not support ATP production required for its survivability. Hess (2014) remarked that buffering capacity of lysine amine groups in hemoglobin of RBCs and bicarbonates producing carbon dioxide which diffuse out of plastic bag prevents pH drop to a critical level. In a study, Rodrigues *et al*. (2020) used canine

Table 2: Variations in biochemical parameters of pRBCs during the storage period

Variable	*Day 0*	*Day 14*	*Day 28*	*Day 42*	*F-value (P-value)*
pH	7.19 ± 3.50	7.00 ± 5.94	6.80 ± 4.92	6.66 ± 3.72	0.005^{ns} (0.999)
Potassium (mM/L)	2.27 ± 3.50	3.38 ± 5.94	4.34 ± 4.92	4.79 ± 3.72	0.126^{ns} (0.944)
Glucose (mg/dL)	624.40 ± 3.50^{a}	528.60 ± 5.94^{b}	421.90 ± 4.92^{c}	333.20 ± 3.72^{d}	1599.797 (0.000)**
GSH (µM/gHb)	4.83 ± 3.50	3.97 ± 5.94	3.20 ± 4.92	2.81 ± 3.72	0.081^{ns} (0.970)
MDA (µmol/L)	44.82 ± 3.50^{a}	57.63 ± 5.94^{b}	91.17 ± 4.92^{c}	120.38 ± 3.72^{d}	104.556 (0.000)**

**-significant at $p < 0.01$ ns- non significant ($p > 0.05$)
Means ± SE having different letters as superscript differ significantly within rows

pRBCs in CPDA-1 (citrate phosphate dextrose adenine) stored for 28 days, and during the storage period pH reduced from 6.98 on 0th day to 6.41 on 28th day. The authors recorded that the low pH of stored pRBCs, when used for transfusion, did not alter the blood pH of canine recipients. It was safer to use CPD-SAGM to store canine pRBCs for 42 days unless massive transfusions are required.

Potassium in Supernatant

Incrementing the level of potassium in the supernatant of pRBCs on storage was in accordance with the findings of Ekiz *et al*. (2012), and Lacerda *et al*. (2014), though it was not statistically significant in the present study. Storage-induced ATP depletion in pRBCs adversely affected membrane transport mechanisms. Cold storage and ATP decline, inactivated cell membrane Na^+-K^+-ATPase pumps and caused leakage of intracellular potassium ions to the supernatant (Adams *et al*., 2015). According to Lehmann *et al*. (2019), potassium could be used as a marker of ATP depletion during the storage of packed RBCs. However, the decline in potassium values is of much lesser magnitude in this study when compared to previous human studies (Nogueira *et al*., 2015). Clinical hyperkalaemia following transfusion could cause life-threatening consequences (Smith *et al*., 2008 and Rizos *et al*., 2017). Relatively low concentration of intracellular potassium (7 mmol/L) compared to humans (45 mmol/L) and a limited number of Na^+-K^+-ATPase pumps on the surface of canine RBCs might be the reason (Wilson *et al*., 2017). Hence high potassium values are of less clinical significance in canine recipients following transfusion of stored RBCs (Rodrigues *et al*., 2020). In contrast, few Japanese and Korean breeds of dogs have high intracellular potassium concentrations (Fujise *et al*., 1997). As storage of RBCs could be associated with accumulation of potassium in supernatant dogs of these breeds were not preferred for blood donation (Tsvilikhovski *et al*., 2018).

Glucose in Supernatant

Mean glucose concentration decreased significantly ($p < 0.05$) from 624.4 ± 3.50 mg/dL (day 0) to 333.20 ± 3.72 mg/dL at day 42. This could be due to the utilization of glucose by erythrocytes as their energy source to produce ATP *via* glycolysis (Hess, 2010). According to Paglia *et al*. (2016), glucose consumption in stored RBCs was a good indicator of metabolic activity of RBCs. The gradual decrease observed in the current study agreed with the previous canine transfusion studies (Ekiz *et al*., 2012; Lacerda *et al*., 2014). Lower values obtained during the study period could be due to excess glucose consumption by leucocytes when compared to leuco reduced pRBC's (Lacerda *et al*., 2014).

Reduced Glutathione

A decrease in GSH concentration was noticed throughout the study period. A decline in GSH level has been reported by Collard *et al*. (2014) and Bardyn *et al*. (2017) in human pRBC studies. Erythrocytes are inherently susceptible to oxidant injury due to oxygen proximity, iron content and lack of nuclear material (Lushchak, 2015). According to Chaudhuri *et al*. (2008), intracellular GSH was major antioxidant defense mechanism of RBCs. Low temperature and progressive decline in pH during storage inhibit the production of nicotinamide adenine dinucleotide phosphate (NADPH). It is a major cofactor of glutathione reductase responsible for producing GSH. In addition to this, *de novo* synthesis of glutathione is ATP-dependent and declines when ATP production falls on storage (Bardyn *et al*., 2017).

Malondialdehyde

Mean malondialdehyde concentration increased throughout storage from 44.82 ± 3.50 µmol/L on day 0 to 120.38 ± 3.72 µmol/L on day 42. The study demonstrated a significant increase in MDA level throughout the storage period. This indicates the degree of oxidative stress in stored RBCs and lipid peroxidation resulting in membrane damage. Malondialdehyde was the most abundant product of lipid peroxidation of RBC membrane (Collard *et al*., 2014). Therefore, it was considered as an excellent marker of oxidative injury in RBC. Oxidative stress is of prime importance to stored RBC as it contributes to storage lesion and ultimately causes loss of erythrocyte deformability, increased rigidity, and enhanced formation of microparticles (Obrador *et al*., 2015). The mean values of MDA markedly increased from day 14 to 28. This is in concurrence with the reports of Herring *et al*. (2013). The authors demonstrated that microparticles, another marker for oxidative stress, were formed in stored canine RBCs after 2 weeks. Lipid peroxidation caused by hydroxyl radicals due to Fenton reaction in stored RBC attack cell membrane fatty acids and alter cell membrane integrity (Ayala *et al*., 2014).

Conclusion

The current study established that significant biochemical alteration occurred during the storage of canine-packed RBCs in CPD-SAGM solution for 42 days. A progressive decline in glucose level indicates the viability of stored RBCs, and decreased glutathione level along with increased potassium and malondialdehyde indicate an oxidative injury and RBC membrane damage.

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