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DYNAMICS OF SOME BIOCHEMICAL INDICATORS IN CANINE PRBC DURING STORAGE PERIOD

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Abstract.*. According to the standards of humane medicine, a large concentration of extracellular potassium may accumulate in the human packed red blood cells (pRBCs) during storage. Therefore, during transfusion to the recipient of such pRBCs, there is a risk of developing hyperkalaemia. This is one of the reasons for a significant restriction of the use of erythrocytic mass in humane medicine, sometimes after two weeks of storage. In veterinary medicine there is no research on the dynamics of accumulation of potassium and lactate in the canine pRBCs. The aim of the study was to determine the concentrations of potassium and lactate in the canine pRBC for different periods of its storage. According to the results of the research, the concentration of potassium during the 35-day storage period of the canine pRBCs significantly increased in 2.1 times from 3.89 ± 0.16 mmol/L to 8.12 ± 0.26 mmol/L, and lactate concentration increased in 10.7 times from 1.8 ± 0.07 mmol/L to 19.3 ± 0.25 mmol/L, followed by a rapid decrease in these parameters after the 21st day of pRBC storage. It was established that the concentrations of potassium and lactate in the canine pRBC on the 35th day of storage is safe for its transfusion to animals. Moreover, the level of lactate in the canine pRBC can be used as a criterion for its suitability for transfusion to patients. In the future, it is important to determine the dynamics of changes in the morphological parameters of the erythrocytic mass of dogs for different periods of its storage.*

Keywords: dogs, packed red blood cells, potassium, lactate, glycolysism phosphofructokinase

Introduction. Biochemical processes of packed red blood cells (pRBCs) are of vital importance for understanding the pathophysiology of morphological changes in erythrocytes during storage and the development of standards for holding time and conditions of blood product storage. According to the standards of humane medicine, a large concentration of extracellular potassium can be accumulated in human pRBCs during storage. Therefore,

the transfusion of such pRBCs to the recipient poses a risk of developing hyperkalaemia. In humane medicine this is one of the reasons for the significant restriction of the use of pRBCs at times after two weeks of storage.

At present, in veterinary medicine there are no studies on the dynamics of potassium accumulation in canine pRBCs. There is also no research on the dynamics of lactic acid (lactate) accumulation in canine erythrocytes at different storage times that can be an important factor in the development of lactic acidosis in recipients during the conduct of massive transfusions.

Aim of the study. The aim of this study was to determine the concentrations of potassium and lactate in canine pRBCs at different storage periods.

Materials and methods. The studies were performed at the Department of Therapy and Clinical Diagnostics, National University of Life and Environmental Sciences of Ukraine and in the department of the animal blood bank, "Zoolux" Veterinary Clinic, Kyiv. In our studies, the canine pRBCs with different storage periods were used.

Packed red cells were obtained according to generally accepted standard techniques using donor dogs 2-7 years of age, which had a body weight of at least 25 kg. Before each blood sampling, the donor dogs underwent a general clinical examination. With satisfactory results of clinical studies, blood was sampled from the donor dogs for babesiosis and microfilaraemia tests. Also, in the blood of each donor dog before donation, the number of erythrocytes and leukocytes and HCT were determined. Blood sampling was carried out from the jugular vein of the animal using special containers with anticoagulant CPDA-1, which includes citric acid, dextrose monohydrate, sodium citrate, sodium dihydrogen phosphate and adenine. The whole blood separation was carried out by centrifugation for 20 minutes at 2500 rpm followed by blood plasma extraction. After the separation, the packet with the pRBCs was marked, noting the identification number of the donor, the date of sampling and the volume. The end of the infusion tubing line was sealed.

According to the recommendations of Animal Blood Resources International, the packet with pRBCs was placed in special refrigerating chambers in a vertical suspended position at a temperature of +1 ... +6°C [1]. Each day packages were examined for haemolysis of erythrocytes, a change in the colour of pRBCs, the appearance of clots, and thoroughly mixed.

Fifteen packets with pRBCs, which was obtained from different donor dogs, were used for the study. Concentrations of potassium and lactate in each sample on the day of pRBC sampling (the 1st day) as well as on the 7, 14, 21, 28 and 35 days of storage were the control points.

Plasma for determining the concentration of potassium and lactate in it was obtained by centrifuging a tube with 2 ml of pRBCs for 10 minutes at 3000 rpm. Determination of potassium and lactate content in plasma was carried out using a semi-automatic biochemical analyzer Statfax 4500.

Results. Potassium and lactate concentrations in the obtained canine pRBCs (the 1st day) were 3.89 ± 0.16 mmol / L (3-5.3 mmol / L) and 1.8 ± 0.07 mmol / L (1.5-2.3 mmol / L), respectively, as shown in Table 1.

1. Potassium and lactate content in the canine pRBCs with different periods of their storage, *M* ± *m*, *n* = 15

Day of pRBCs storage period	Potassium, mmol/L			Lactate, mmol/L		
	general	difference		general	difference	
		as compared with previous result	as compared with the 1st day		as compared with previous result	as compared with the 1st day
1	3.89 ± 0.16	-	-	1.8 ± 0.07	-	-
7	4.4 ± 0.16*	0.51	0.51	7.2 ± 0.21***	5.4	5.4
14	5.07 ± 0.21***	0.67	1.18	12.1 ± 0.2***	4.9	10.3
21	6.04 ± 0.26**	0.97	2.15	16.3 ± 0.25***	4.2	14.5
28	7.17 ± 0.3***	1.13	3.28	18.2 ± 0.22***	1.9	16.4
35	8.25 ± 0.26***	1.08	4.36	19.3 ± 0.25***	1.1	17.5

Notes: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001 in comparison with the 1st day of pRBCs storage

On the 7th day of canine pRBC storage, the concentration of potassium increased 1.13 times and was 4.4 ± 0.16 mmol / L (3.2-5.6 mmol / L), and the concentration of lactate increased 4.0 times and was 7.2 ± 0.21 mmol / L (5.9-8.5 mmol / L).

On the 14th day of storage, the concentrations of potassium and lactate in the canine pRBCs were 5.07 ± 0.21 mmol / L (3.9-7.1 mmol / L) and 12.1 ± 0.2 mmol / L (11.1-13.5 mmol/L), respectively.

On the 21st, 28th and 35th days of the pRBC storage, the concentration of potassium in it increased to 6.04 ± 0.26 mmol / L (5-8.9 mmol / L), 7.17 ± 0.3 mmol / L (5.1-9.4 mmol / L) and 8.25 ± 0.26 mmol / L (7.0-10.6 mmol / L), respectively.

But on the 21st, 28th and 35th days of pRBC storage the concentration of lactate in it was 16.3 ± 0.25 mmol / L (14.9-18.0 mmol / L), 18.2 ± 0.22 mmol / L (17.2-20.2 mmol / L) and 19.3 ± 0.25 mmol / L (18.0-20.9 mmol / L), respectively (see Table 1).

Thus, during the period from the 1st to 7th days, the concentration of potassium in the canine pRBCs increased by 0.51 mmol / L; from the 7th to 14th days by 0.67 mmol / L; from the 14th to 21st days by 0.97 mmol / L; from the 21st to 28th day by 1.13 mmol / L; and from the 28th to 35th days by 1.08 mmol / L (Table 1, Fig. 1).

From the 1st to 7th day, the concentration of lactate in the canine pRBCs increased by 5.4 mmol / L in comparison with the previous measurement; from the 7th to 14th days by 4.9 mmol / L; from the 14th to 21st days by 4.2 mmol / L; from the 21st to 28th day by 1.9 mmol / L; from the 28th to 35th day by 1.1 mmol / L (Table 1, Fig. 2).

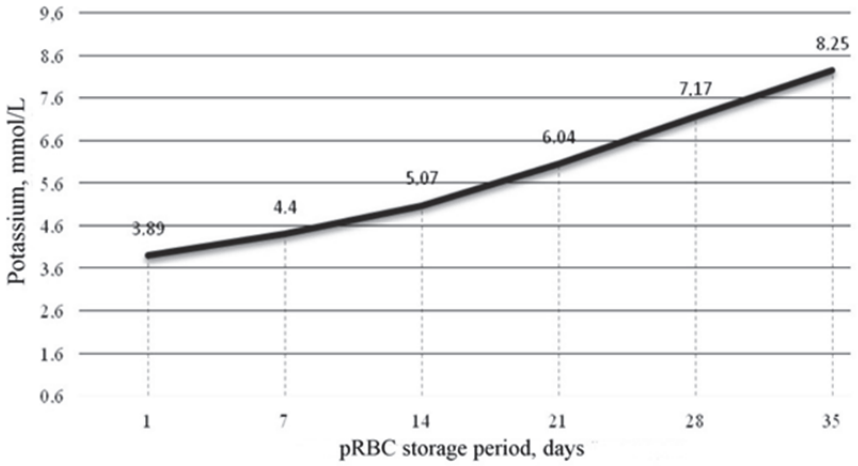

Fig. 1. Dynamics of potassium content growth in canine pRBCs during storage, mmol / L, *n* = 15

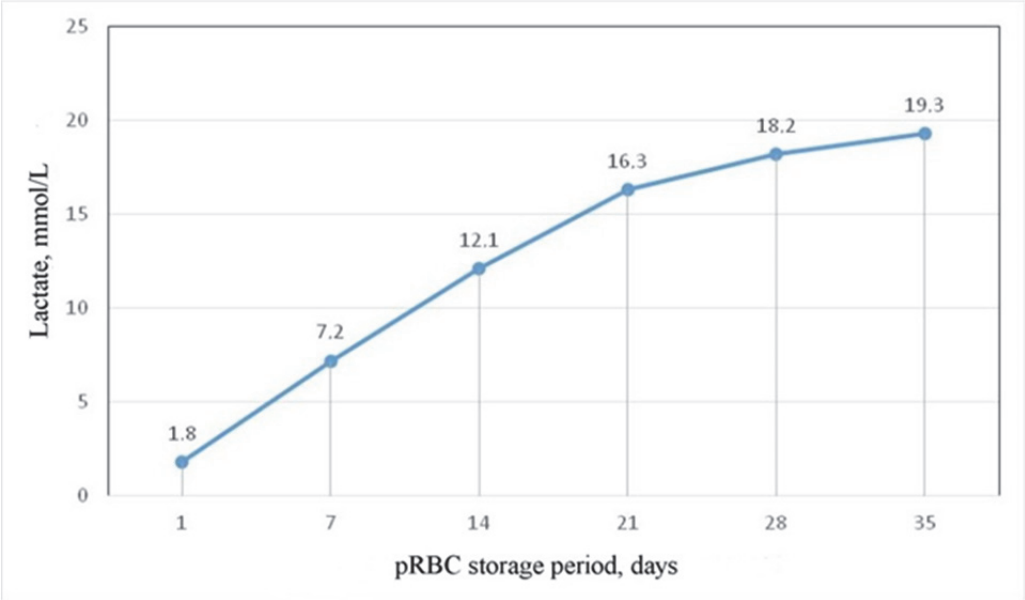

Fig. 2. Dynamics of lactate content growth in canine pRBCs during storage, mmol / L, *n* = 15

To maintain shape, ability to deform, phosphorylate membrane phospholipids and proteins, provide transport function of membranes, synthesize purine and pyrimidine nucleotides, and synthesize glutathione, erythrocytes need energy in the form of ATP [4].

Mature erythrocytes have no mitochondria, so the only source of energy for them is the anaerobic breakdown of glucose (glycolysis) using the Embden–Meyerhof pathway. During this process, glucose is converted to pyruvate, then followed by lactate, which results in the formation of 2 molecules of ATP and lactate per each glucose molecule.

An enzyme that catalyzes the conversion of fructose-6-phosphate to fructose 1,6-diphosphate and regulates the rate of glycolysis in circulating red blood cells is phosphofructokinase (PFK) [5]. ATP is not only a substrate but also an inhibitor of PFK. When the hydrolysis of ATP is slower than its

synthesis, it is attached to the allosteric center of PFK and reduces its affinity for fructose-6-phosphate. The concentration of ADP and AMP increases in the event of intensive use of ATP. They act as activators, weakening the effect of ATP on PFK-1. Thus, the activity of phosphofructokinase is regulated in all tissues, including in erythrocytes. Moreover, when blood is stored, the dominant inhibitor of PFK is the activity of hydrogen ions, the level of which increases in the course of accumulating lactic acid in pRBCs [6].

Due to prolonged inhibition of PFK and glycolysis by active hydrogen ions, red blood cells can produce a sufficient amount of ATP to meet metabolic needs. As soon as the concentration of ATP decreases, the activity of erythrocyte K^+-Na^+ ATPase, the stability of membrane, the processes of glucose transport, the level of protective mechanisms from oxidative stress and the distribution of membrane phospholipids also sharply decrease [5]. This can lead to the destruction of the phospholipid membrane of erythrocytes, their oedema and the release of free haemoglobin.

Because of the rapid loss of ATP followed by the inactivation of K^+-Na^+ATPase in human erythrocytes, a rapid increase in potassium content in the supernatant of pRBCs is observed after 14 days. Transfusion of the pRBCs with a high content of potassium to the recipient can lead to the appearance of cardiac arrhythmias, and sometimes to the death of the patient [8]. However, in the process of canine pRBC storing, based on the results of our studies, the potassium level in most cases has a small growth rate. The highest level of potassium we observed in the canine pRBCs was 10.6 mmol / L. Given that the ordinary maintenance infusion solutions should have a potassium content of 15-30 mmol / L, the potassium content obtained in our test samples makes it possible to conclude that transfusion of the pRBCs even with a 35-day storage period will not cause a clinically pronounced hyperkalaemia in the animal [2, 3]. An exception may be patients who have hyperkalaemia even before carrying out blood transfusion, e.g. in the event of anuria or severe acidotic conditions. Significant electrolyte disorders can occur only during massive blood transfusions in the event of transfusion of 50 % or more of the circulating blood volume.

A slight increase in the potassium content in the pRBCs at different storage periods confirms the low potassium concentration and low Na^+-K^+ATPase activity in the erythrocyte plasmalemma in dogs [7]. However, dogs of some breeds (Japanese and Korean breeds) have a high level of potassium in erythrocytes due to high activity of Na^+-K^+ATPase. In the process of obtaining pRBCs from donor dogs belonging to these breeds and further pRBC storage there is a high risk of rapid buildup of the level of potassium in the pRBCs. Thus, in the studies performed it was found that potassium concentration in erythrocytes of these breeds can be up to 70 mmol/L, and the development of haemolysis when storing their pRBCs leads to a rise in the level of potassium in plasma up to 24 mmol / L [9]. Therefore, dogs of these breeds should not be employed for the donor programme.

The accumulation of lactate in the pRBCs makes it possible to draw conclusions about its energy level. As can be seen from the results of our studies,

the greatest rise in lactate concentration in the pRBCs is observed during the first three weeks of their storage. It should be noted that the dynamics of growth of lactate concentration in the canine pRBCs slows down on weekly basis. As indicated above, the intensity of glycolysis in erythrocytes is regulated by the level of hydrogen ions at the expense of the concentration of lactic acid. With a decrease in the level of ATP in the pRBC, the activity of phosphofructokinase increases, as a result of which glycolysis is increased. This, in turn, leads to a rise in lactate concentration, which gradually inhibits phosphofructokinase and reduces the rate of glycolysis. Rapid growth of lactate concentration in the canine pRBCs after 3 weeks of storage indicates a cessation of glycolysis processes, a catastrophic deficiency of ATP, and the onset of destruction of the cytoplasmic membrane of erythrocytes. At the same time, the lactate concentration in the canine pRBC at the level of 19.3 mmol / L even on the 35th day of the study is safe for transfusion it to the majority of critical patients because the level of lactate in the isotonic Ringer-lactate solution, which is a solution for maintenance around-the-clock infusion, is 28 mmol / L.

Conclusions

1. During the storage of canine pRBCs, the concentration of potassium tends to raise. However, the concentration of potassium at the level of 8.25 ± 0.26 mmol / L in canine pRBCs at the 35-day storage period in most cases will not cause significant electrolyte disturbances in the recipients, which is explained by the low activity of the erythrocyte plasmalemma Na^+-K^+ATPase in the majority of dogs.

2. The concentration of lactate rapidly increases in the canine pRBCs during the first three weeks of storage, after which the dynamics of growth significantly declines. This indicates the cessation of metabolic processes in erythrocytes and the onset of destruction of their plasma membranes. The concentration of lactate in the pRBCs even on the 35th day of its storage (19.3 ± 0.25 mmol / L) does not exceed the lactate content in solutions used for twenty-four-hour infusion therapy of patients.

Prospects for further studies. Further studies on the dynamics of changes in morphological parameters of erythrocytes in the process of storing canine pRBCs and conducting clinical studies to determine the safety and effectiveness of blood transfusion to recipients using canine pRBCs with different storage periods are promising.

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ДИНАМІКА ДЕЯКИХ БІОХІМІЧНИХ ПОКАЗНИКІВ ЕРИТРОЦИТАРНОЇ МАСИ СОБАК ПІД ЧАС ЗБЕРІГАННЯ

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Анотація. Згідно стандартів гуманної медицини, в еритроцитарній масі людини під час зберігання може накопичуватися велика концентрація позаклітинного Калію. Тому під час трансфузії реципієнту такої еритроцитарної маси існує ризик розвитку гіперкаліємії. Це є однією з причин значного обмеження використання еритроцитарної маси у гуманній медицині інколи вже через два тижні зберігання. У ветеринарній медицині немає досліджень щодо динаміки накопичення Калію і лактату в еритроцитарній масі собак. Метою дослідження було визначення концентрації Калію та лактату в еритроцитарній масі собак за різних термінів її зберігання. В результаті досліджень встановлено, що концентрація Калію впродовж 35-добового терміну зберігання еритроцитарної маси собак достовірно збільшилася в 2,1 рази з $3,89 \pm 0,16$ ммоль / л до $8,12 \pm 0,26$ ммоль / л, а лактату – в 10,7 разів з $1,8 \pm 0,07$ ммоль / л до $19,3 \pm 0,25$ ммоль / л з подальшим швидким зниженням вказаних показників після 21 доби зберігання еритроцитарної маси. Встановлено, що концентрація Калію та лактату в еритроцитарній масі собак на 35 добу зберігання є безпечною для її трансфузії тваринам. Більше того, рівень лактату в еритроцитарній масі собак може використовуватися як критерій її придатності для трансфузії пацієнтам. На перспективу важливим є визначення динаміки змін морфологічних показників еритроцитарної маси собак за різних термінів її зберігання.

Ключові слова: собаки, еритроцитарна маса, калій, лактат, гліколіз, фосфофруктокіназа

ДИНАМИКА НЕКОТОРЫХ БИОХИМИЧЕСКИХ ПОКАЗАТЕЛЕЙ ЭРИТРОЦИТАРНОЙ МАССЫ СОБАК ВО ВРЕМЯ ХРАНЕНИЯ

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Аннотация. *Согласно стандартам гуманной медицины, в эритроцитарной массе человека во время хранения может накапливаться большая концентрация внеклеточного Калия. Поэтому во время трансфузии реципиенту такой эритроцитарной массы существует риск развития гиперкалиемии. Это является одной из причин значительного ограничения использования эритроцитарной массы в гуманной медицине иногда уже через две недели хранения. В ветеринарной медицине нет исследований по динамике накопления Калия и лактата в эритроцитарной массе собак. Целью исследования было определение концентрации Калия и лактата в эритроцитарной массе собак при различных сроках ее хранения. В результате исследований установлено, что концентрация Калия в течение 35-дневного срока хранения эритроцитарной массы собак достоверно увеличилась в 2,1 раза – с 3,89 ± 0,16 ммоль / л до 8,12 ± 0,26 ммоль / л, а лактата – в 10,7 раз с 1,8 ± 0,07 ммоль / л до 19,3 ± 0,25 ммоль / л с последующим быстрым снижением указанных показателей после 21 дня хранения эритроцитарной массы. Установлено, что концентрация Калия и лактата в эритроцитарной массе собак на 35 сутки хранения является безопасной для ее трансфузии животным. Более того, уровень лактата в эритроцитарной массе собак может использоваться как критерий ее пригодности для переливания пациентам. В перспективе важным является определение динамики изменений морфологических показателей эритроцитарной массы собак при различных сроках ее хранения.*

Ключевые слова: собаки, эритроцитарная масса, калий, лактат, гликолиз, фосфофруктокиналаза