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CANGES IN THE CONCENTRATION OF 2,3 DPG AND METHEMOGLOBIN IN CANINE BLOOD DURING PRESERVATION

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In order to investigate the quality of preserved canine blood, in our work, we determined 2,3 diphosphoglycerate (DPG) concentrations, as well as methemoglobin concentrations in erythrocytes of acid-citrate - dextrose (ACD) blood.

During storage, the 2,3 DPG concentration in erythrocytes was highest on day 0, amounting to 2.615 ± 0.152 mmol/L. On day 7 of storage the 2,3 DPG concentration was significantly lower, being 2.297 ± 0.142 mmol/L ($p<0.01$). The 2,3 DPG concentrations found on days14 and 21 were even lower and that day o the and that between them being highly significant ($p<0.001$).

Determination of methemoglobin demonstrated a significant rise in the level of this hemoglobin derivative in canine blood during preservation. For example on day 5 of storage the level of methemoglobin was 1.486 ± 0.162g/L, which was a significant increase as compared to the initial concentration of 1.327 ± 0.155 g/L. The highest level of methemoglobin in canine blood was found on day 21 of storage, when it amounted to 2.108 ± 0.280 g/L ($p<0.001$).

The data acquired show that the possibility of using blood older than 14 days, for massive transfusions significantly decreases, due to a significant rise in the concentration of methemoglobin and a drop in the concentration of 2,3 DPG in the erytrocytes.

Key words: preserved canine blood, ACD solution, 2,3 DPG, methemoglobin.

INTRODUCTION

The affinity of hemoglobin towards oxygen may be attered through a change in the concentration of 2,3 diphosphoglycerate (2,3 DPG) in the erythrocytes. Thus, the discovery of the effect of 2,3 DPG on the function of hemoglobin and better knowledge of the molecular physiology and biochemistry of erythrocytes have enabled clarification of the mechanism of oxygen transport, i. e. study of tissue oxygenation in various physiological and pathological states.

The compound 2,3 DPG can be found the erythrocytes of most mammals. The highest levels are present in camel and lama erythrocytes, while it represents around 60% of all acid-soluble phosphates in human red blood cells. In a biochemical sense, 2,3 diphosphoglycerate is a metabolic intermediary intermediary on the main glycolytic pathway of the erythrocyte (Walker, 1973).

The concentration of 2,3 DPG in erythrocytes depends primarily on the activity of two enzyme systems: diphosphoglycerate mutase (DPG-mutase) and diphosphoglycerate phosphatase (DPG-phosphatase). The concentration of 2,3 DPG is also influenced by the enzyme phospho-fructo-kinase, as well as the ratio between ATP and ADP. In erythrocytes, 2,3 DPG is found free or bound to hemoglobin, depending on whether the hemoglobin is in a deoxy or an oxy form. Namely, research of Walker (1973) established that 2,3 DPG positions and binds to hemoglobin between two beta chains in the deoxy conformation.

When oxygen is bound to hemoglobin 2,3 DPG is released, i. e. squeezed out of the deoxyhemoglobin molecule, which leads to an increase in the concentration of its free form in red blood cells, and vice versa, by the binding of 2,3 DPG to hemoglobin, oxygen is releasd. In preserved blood, during storage, the level of 2,3 DPG decreases, and therefore the ability to release oxygen to tissues also decreases.

MATERIALS AND METHODS

A total of 11 bottles of acid-citrate-dextrose (ACD) blood were examined. The donors were German Shepherd dogs, aged 2 to 2.5 years. The blood was preserved in bottles of 125 ml volume, with a sterile and non-pyrogenic ACD solution. During the entire investigation the bottles containing blood were stored at $+4^0$C.

2,3 DPG was determined on the day the blood was drawn (day 0), as well as on days 7,14 and 21. A method using a commercial reagent kit (Boehringer Manheim) was used as prescribed by the manufacturer. The initial and final absorption (at 340 nm) was read using a "Dr Lange" LP 300 S spectrophotometer.

Methemoglobin concentrations in preserved canine blood were determined continuously from day 0 to day 21 of storage using an automatic

"CO-oximeter 2500", Corning Diagnostics Corp. analyzer.

RESULTS AND DISCUSSION

Table 1 show the values of 2,3 diphosphoglycerate concentration found in erythrocytes from the investigated blood samples during the period of blood preservation which lasted 21 days.

Table 1. Changes of 2,3 DPG concentration in preserved canine blood (mmol/L)

Day	n = 11					
	$\bar{x}$	SD	SE	CV %	t	p
00	2.615	0.152	0.048	5.829	–	–
01	2.452	0.164	0.052	6.699	2.303	<0.05*
07	2.297	0.142	0.045	6.173	4.833	<0.001***
14	2.082	0.183	0.058	8.783	7.080	<0.001***
21	1.902	0.225	0.071	11.840	8.319	<0.001***

These results indicate that as early as day 1 of storage there was a significant drop in 2,3 DPG concentration (day 0: 2.615 ± 0.151 mmol/L; day 1: 2.452 ± 0.164 mmol/L; $p<0.05$). The most pronounced drop in 2,3 DPG concentration (13%) occurred during the first 7 days of blood preservation, while later on the 2,3 DPG concentration continued to decrease, but more slawly. Thus, compared to day 0, the percentage drop in concentration on day 1 was 6%, on day 7 - 12% on day 14 - 21%, and on day 21-27%. We are especially stressing this since if the decrease in 2,3 DPG concentration on day 7 was 12%, it might be expcted that on day 21 it would amount to 36 %. These data considerably differ from those established for preserved human blood.

For example, according to the investigations of Walker (1973) and Agranenko and cowarkers (1977), the 2,3 DPG concentration in conserved human red blood cells drops very rapidly at the end of the first week. Morever, Radović et al. (1978) found that in erythrocytes of human ACD blood there is a rapid drop in the concentration of 2,3 DPG during the first twelve days of preservation. After day 12, the decline is milder.

Enoki and cowarkers (1986) found that the 2,3 DPG concentration in canine blood which was stored in ACD solution at + 4^0C, decreased much more slowly than in human blood.

In addition, Eisenbrandt and Smith (1973) noticed a significant drop in 2,3 DPG concentration in ACD canine blood after two weeks, and in CPD blood after four weeks. It appears that canine blood preserved in CPD is more efficient concerning the transport of oxygen to tissues.

Although our investigations were primarily aimed at establishing and monitoring changes of 2,3 DPG concentrations in erythrocytes of ACD canine blood, they enabled us to establish normal values for 2,3 DPG concentrations in canine erythrocytes. Namely, the concentration on day 0 (2.615 ± 0.152 mmol/L) should be considered as the normal concentration.

The values found for methemoglobin concentrations in preserved canine blood are presented in Table 2.

Table 2. Concentration of methemoglobin in preserved canine blood (g/L)

Day	n = 11					
	$\bar{x}$	SD	SE	CV %	t	p
00	1.327	0.155	0.049	11.659	–	–
01	1.317	0.105	0.033	7.989	0.169	>0.05
02	1.411	0.137	0.043	9.711	1.289	>0.05
03	1.423	0.124	0.039	8.749	1.533	>0.05
04	1.431	0.119	0.038	8.289	1.677	>0.05
05	1.486	0.162	0.051	11.929	2.248	<0.05*
06	1.667	0.138	0.044	8.303	5.163	<0.001***
07	1.770	0.131	0.041	7.406	6.934	<0.001***
08	1.811	0.103	0.033	5.707	8.193	<0.001***
09	1.828	0.125	0.040	6.839	7.921	<0.001***
10	1.855	0.119	0.038	6.439	8.515	<0.001***
11	1.884	0.126	0.040	6.705	8.806	<0.001***
12	1.885	0.171	0.054	9.078	7.652	<0.001***
13	1.902	0.157	0.050	8.259	8.213	<0.001***
14	1.911	0.144	0.046	7.532	8.689	<0.001***
15	1.993	0.135	0.043	6.784	10.216	<0.001***
16	1.991	0.177	0.056	8.884	8.923	<0.001***
17	2.030	0.178	0.056	8.792	9.448	<0.001***
18	2.073	0.225	0.071	10.864	8.648	<0.001***
19	2.078	0.284	0.090	13.659	7.329	<0.001***
20	2.086	0.276	0.087	13.211	7.601	<0.001***
21	2.108	0.280	0.088	13.274	7.754	<0.001***

The results obtained indicate that a significant increase in the level of this hemoglobin derivate appears in blood on day 5 of preservation. Namely, the value of 1.486 ± 0.162 g/L, is a significant increase as compared to the initial concentration of 1.327 ± 0.155 g/L. Moreover, the level of methemoglobin in blood increase d constantly with storage duration, so that the highest concentration occurred on day 21 of preservation (2.108 ± 0.280 g/L; $p < 0.001$).

In human trials, Uchida et al. (1990) also found that during the preservation period there is an increase in methemoglobin concentration. The authors recommend that blood containing high methemoglobin concentrations should not be used in massive quantities for transfusions since there is danger of undesirable reactions in critically ill patients.

The significant increase of methemoglobin concentration in canine blood during preservation, which was established here, parallels that decribed also in preserved blood from humans and rats (Sato et al., 1990). Taking into consideration that methemoglobin does not transport oxygen, and considering the recommendations of Uchida et al. (1990), we suggest that in dogs as well one should avoid the use of massive quantities of preserved blood older than two weeks because of high methemoglobin concentrations.

CONCLUSION

In the erythrocytes of preserved blood 2,3 DPG concentration on days 7,14 and 21 is significantly lower than that in erytrocytes from fresh blood.Thus, on day 21 of storage, the 2,3 DPG concentration in erythrocytes was 27.27% lower than the value determined on day 0.

In canine blood preserved in ACD solution at + 4^0C the methemoglobin level continuously and significantly increased up to day 21 of preservation.

The data obtained for the investigated parameters, which represent the biological value of preserved canine blood, show that until day 14 of storage preserved blood may be used in unlimited quantities, while blood from day 14 to 21 of storage may be used, but in smaller quantities, due to a considerable increase in methemoglobin concentration and a drop in the 2,3 DPG level.

REFERENCES

1. *Agranenko, V. A., V. L. Golubeva.* 1977. Gematol Pereliv Krovi, *22,6.*
2. *Esenbrandt, D. L. and Smith, J.* 1973. Use of biochemical measures to estimate viability and red blood cells in canine blood stored in acid citrate dextrose solution, with and without added ascorbic acid. *J. A. V. M. A., No. 8,* 984-987.
3. *Enoki Y., Watanabe T., Ohga Y.* Posttransfusional recovery of defective respiratory function of stored blood in dogs. *Jpn J Phisiol, 36 (6),* 1125-39,
4. *Radović M., Đurić D., Taseski J., Todorović P., Ivanović I., Miljušković Z., Milenković Lj.,* 1978. Koncentracija 2,3 DPG u eritrocitima ACD krvi. *Zbornik vojnomedicinske akademije, Beograd,* 56-59.
5. *Sato, K., Tamaki, K., Tsutsumu, H., Okajima, H., Katsumata, Y.* 1990. Storage of blood for methemoglobin determination-comparison of storage with a crioprotectant at-30 degrees C and additions at 80 degrees C or-196 degrees C. *Forensik Science International, 45.* 1-2, 129-34.
6. *Uchida, I., Tashiro, Ko Y. H., Mashimo T., Yoshiya, I.* 1990, Carboxyhemoglobin and methemoglobin levels in banked blood. *Journal of Clinical Anesthesia, 2,* 86-90.
7. *Walker, G.* 1973. Ninth Symposium on Advanced Medicine, London, Pitman Medical.

PROMENE KONCENTRACIJE 2,3 DPG I METHEMOGLOBINA U KRVI PASA TOKOM KONZERVISANJA

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SADRŽAJ

U uzorcima konzervisane krvi pasa u ACD rastvoru, čuvane 21 dan na +4^0C ispitivana je koncentracija 2,3 DPG i koncentracija methemoglobina.

Na osnovu dobijenih rezultata zaključeno je da se konzervisana krv do 14. dana čuvanja može koristiti u neograničenim količinama, a krv od 14. do 21 dana čuvanja, zbog značajnog povećanja koncentracije methemoglobina i opadanja nivoa 2,3 DPG-a je upotrebljiva ali u manjim količinama.