SHORT REPORTS

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ELECTROLYTE STATE OF CANINE RED BLOOD CELLS DURING HYPOTHERMIC STORAGE WITH THE ADDITION OF N-ACETYLCYSTEINE

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Aim. To evaluate the effect of adding N-acetylcysteine (NAC) to SAGM (adenine-glucose-mannitol saline) solution on the electrolyte composition and pH of canine erythrocytes during hypothermic storage.

Material and Methods. Red blood cells were stored in SAGM solution with or without NAC at 4–5 °C. The concentrations of sodium, potassium, chloride, and pH were determined on days 0, 7, 21, and 35 of storage.

Results. The sodium concentration increased in both groups, slightly less in the experimental group with NAC. The accumulation of potassium was less pronounced in the NAC-supplemented group. Chloride levels remained stable, and pH decreased, particularly in the experimental group.

Conclusions. NAC contributes to the stabilization of the electrolyte environment during storage, in particular to potassium retention and pH control.

Key words: erythrocytes, hypothermic storage, electrolyte balance, N-acetylcysteine, antioxidant protection.

Hypothermic storage of red blood cells is an essential practice in veterinary transfusion medicine, allowing for the long-term preservation of donor blood. Despite the effectiveness of this approach, storage at 4–5 °C leads to a gradual deterioration of red blood cell integrity due to metabolic changes and oxidative stress [1]. During storage, red blood cells undergo changes in volume, shape, membrane deformation, and metabolic profile, including progressive ATP depletion and lactate accumulation, which impair their functionality. When choosing red blood cell storage conditions, maintaining an optimal pH level and regulating electrolyte gradients play an essential role. These parameters affect the efficiency of gas exchange and provide the necessary conditions for the stability of red blood cell function [2].

Potassium (K^+) is the principal intracellular cation and plays a fundamental role in maintaining cell membrane potential, osmotic balance, and enzymatic activity. During storage, erythrocytes tend to lose K^+ to the extracellular environment due to changes in membrane permeability and energy depletion.

Sodium (Na^+), which is usually maintained at a low level within canine red blood cells, tends to accumulate during storage, altering the osmotic balance and indicating membrane instability. Chloride (Cl^-), a key anion, is also involved in regulating pH and fluid balance.

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A decrease in pH during storage leads to the accumulation of lactate and a reduction in buffer capacity, which negatively affects the enzymatic function and conformation of proteins in red blood cells [3]. Therefore, monitoring these parameters is essential for assessing the quality of storage.

N-acetylcysteine (NAC), an antioxidant and glutathione precursor, can help stabilize red blood cell membranes and buffer the intracellular environment. NAC acts as a thiol group donor, reducing oxidative stress and maintaining the structure of membrane proteins. This study evaluates its effect on the electrolyte profile and pH of canine erythrocytes stored for 35 days.

This study aimed to evaluate the effect of adding N-acetylcysteine (NAC) to SAGM (adenine-glucose-mannitol saline) resuspension medium on the electrolyte balance (Na^+, K^+, Cl^-) and pH of canine erythrocytes during 35-day hypothermic storage.

Material and Methods

Whole blood samples were collected from clinically healthy donor dogs and processed within 2 hours of collection. Red blood cells were obtained by centrifugation at 5000g for 7 minutes at 4 °C using blood bags equipped with satellite storage containers. The plasma was separated and frozen, and the erythrocyte mass was resuspended in a standard SAGM solution. The experimental group was supplemented with NAC at a specific concentration, while the control group was kept in standard SAGM without NAC.

All samples were stored at 4–5 °C in a blood storage refrigerator. Aliquots for analysis were taken on days 0, 7, 21, and 35 of storage.

Biochemical parameters, including the concentration of sodium (Na^+), potassium (K^+), chlorine (Cl^-), and pH, were evaluated using an EL-5 electrolyte analyzer (Quertimed, Ukraine).

The results were statistically processed using the Statgraphics software package (Manugistic Inc.; Statistical Graphics System, USA). The data were presented in the format M ± SE (mean ± standard error). Each series of experiments was performed at least five times.

Results and Discussion

Electrolyte analysis of the resuspension medium revealed dynamic changes during 35 days of hypothermic storage of erythrocytes. The sodium concentration increased progressively in both the experimental and control groups. On day 0, the Na^+ level was 159.6 ± 1.3 mmol/l in the experimental group and 157.6 ± 1.0 mmol/l in the control group. On day 35, these values increased to 165.4 ± 0.7 mmol/l and 167.75 ± 2.6 mmol/l, respectively. However, these differences were not statistically significant, indicating that NAC had no marked effect on sodium accumulation.

A more pronounced difference was observed in the concentration of potassium. In the experimental group, K^+ increased from 3.48 ± 0.3 mmol/l to 3.75 ± 0.21 mmol/l within 35 days, while in the control group, it increased from 3.51 ± 0.26 mmol/l to 4.59 ± 0.26 mmol/l. This statistically significant increase ($P < 0.05$) in the control group reflects a more substantial leakage of potassium, probably due to membrane instability. NAC supplementation seems to attenuate this effect, indicating improved membrane integrity.

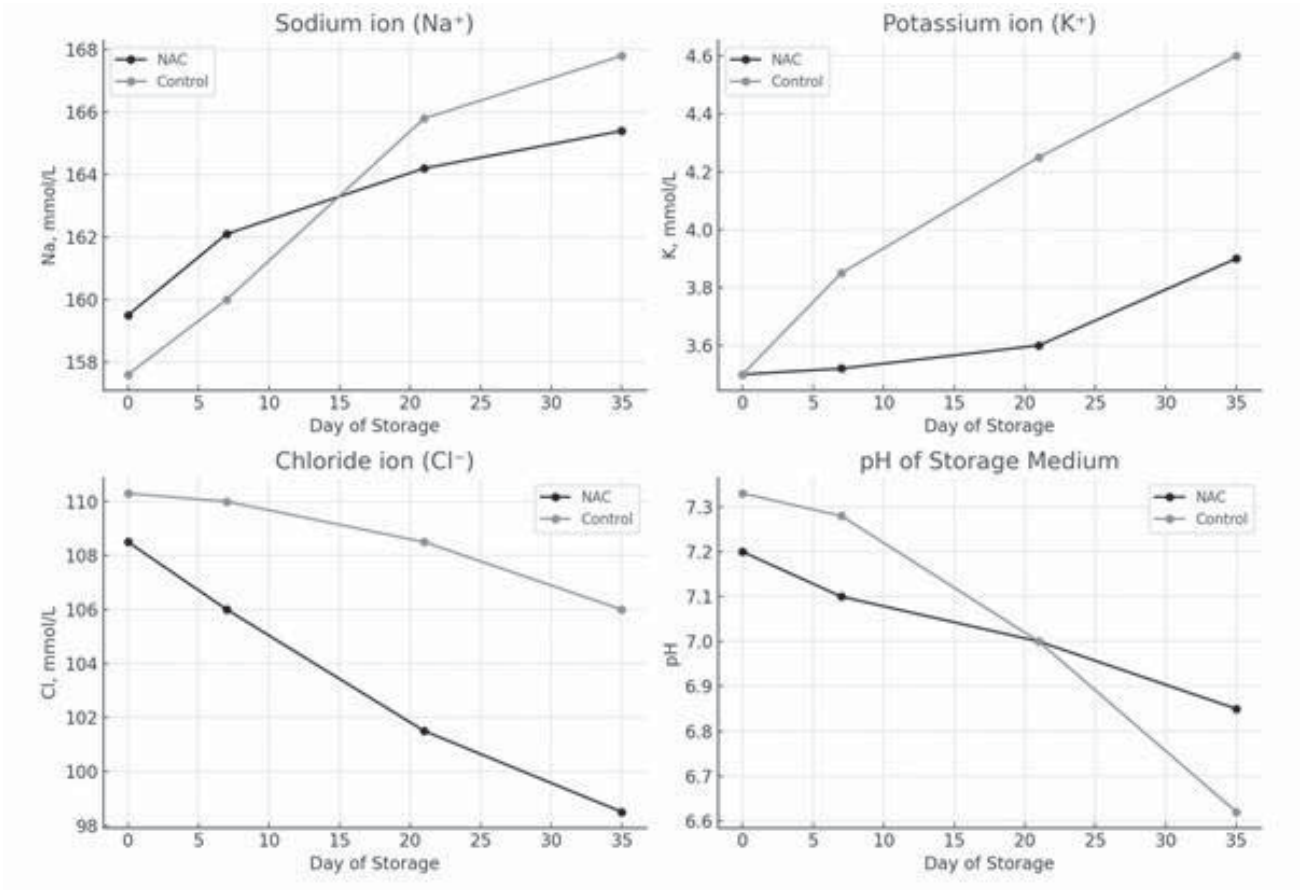
During the 35-day hypothermic storage, the chloride concentration in the resuspension medium gradually decreased in both NAC-treated and control samples. However, no statistically significant differences were found between the groups, indicating that the Cl^- dynamics remained relatively stable and within the physiological range during storage. The pH of the resuspending medium showed a steady downward trend in both groups, indicating acidification due to red blood cell metabolism. In particular, the pH value decreased from 7.21 ± 0.09 to 6.77 ± 0.24 in the NAC group and from 7.35 ± 0.07 to 6.62 ± 0.025 in the control group. Although acidification was observed in both groups, the presence of NAC helped to maintain a relatively higher pH level on day 35.

These results confirm that the addition of NAC can reduce the degree of electrolyte imbalance caused by storage, in particular by limiting potassium efflux and slowing the pH decline. This highlights its potential role in improving the quality of red blood cell storage for clinical use.

The data on changes in Na^+, K^+, Cl^-, and pH levels over time are shown in the Figure.

Conclusions

During 35 days of hypothermic storage, erythrocytes suspended in the SAGM medium showed progressive changes in electrolyte composition and pH. The addition of NAC helped to stabilize the level of Potassium,



Changes in the ionic composition of the SAGM resuspension medium with and without NAC on days 0, 7, 21 and 35

significantly reducing its leakage compared to the control group. Sodium levels increased in both groups, but the addition of NAC slowed the rate of accumulation, indicating improved membrane stability. Chloride concentrations remained within the physiological range, regardless of treatment.

The presence of NAC mitigated the acidification of the storage medium, with pH values decreasing less than in the control group.

These results support the potential use of NAC as a protective agent to improve electrolyte balance and maintain pH in stored red blood cells. The results highlight the potential of NAC as an effective additive to improve the quality and shelf life of red blood cells in veterinary transfusion practice.

Authors' contribution

KRH was responsible for the design of the experiment, preparation of red blood cell samples, data collection, and statistical analysis.

OMD supervised the study and participated in the interpretation of the results and critical revision of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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ЕЛЕКТРОЛІТНИЙ СТАН ЕРИТРОЦИТІВ СОБАКИ ПІД ЧАС ГІПОТЕРМІЧНОГО ЗБЕРІГАННЯ З ДОДАВАННЯМ *N*-АЦЕТИЛЦИСТЕЇНУ

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Мета. Оцінити вплив додавання N-ацетилцистеїну (NAC) до розчину SAGM (аденін-глюкозо-манітол-сольовий розчин) на склад електроліту та pH еритроцитів собак під час гіпотермічного зберігання.

Матеріали та методи. Еритроцити зберігали в розчині SAGM з NAC або без нього за температурою 4–5 °C. Концентрації натрію, калію, хлориду та pH визначали на 0, 7, 21 та 35 добу зберігання.

Результати. Концентрація натрію в обох групах була підвищена, дещо менше в експериментальній групі з NAC. Накопичення калію було менш вираженим у групі, яка отримувала NAC. Рівень хлориду залишався стабільним, а pH знизився, особливо в експериментальній групі.

Висновок. NAC сприяє стабілізації електролітного середовища під час зберігання, зокрема утриманню калію та контролю pH.

Ключові слова: еритроцити, гіпотермічне зберігання, електролітний баланс, N-ацетилцистеїн, антиоксидантний захист.