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Original Research Paper

THE EFFECT OF HIGH ANTICOAGULANT K_3-EDTA CONCENTRATION ON COMPLETE BLOOD COUNT AND WHITE BLOOD CELL DIFFERENTIAL COUNTS IN HEALTHY BEAGLE DOGS

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Summary: The sodium or potassium salts of ethylenediaminetetraacetic acid (EDTA), anticoagulants recommended for routine haematological analyses, are known to have deleterious effects on platelets, erythrocytes and leukocyte counts even at recommended concentrations (1,8 mg/mL of blood). Under-filling of tubes containing K_3EDTA due to difficulties in sampling that produces higher K_3EDTA concentrations than recommended may occur in severely hypotensive, restless, very small or obese dogs and cats. The aim of the present study was to investigate the effect of high anticoagulant K_3EDTA concentration on complete blood count (CBC) and white blood cell differential count (WCDC) in healthy beagle dogs. The recommended volume of 3 and 1 mL of blood were put in K_3EDTA containing tubes to obtain recommended (1.8 mg/mL) and high (5.4 mg/mL) K_3EDTA concentrations (under-filled tubes), respectively. CBC and WCDC were determined with a laser haematology analyser with species-specific software. Presence of platelet clumps was determined by examination of blood smears. The results showed a significant (P<0.05) decrease in hematocrit (HCT) from 0.55 ± 0.043 to 0.48 ± 0.042 (13.4 ± 4.8% decrease) and mean corpuscular volume (MCV) from 74.4 ± 1.1 to 65.4 ± 3.0 fL (11.9 ± 4.2% decrease), and a significant increase in mean corpuscular haemoglobin concentration (MCHC) from 306.5 ± 5.7 to 349.7 ±17.8 g/L (14.0 ± 5.9% increase) in samples with high K_3EDTA concentration in comparison with the recommended concentration. No platelet clumping was detected in all examined blood smears. According to the results of our study, we recommend respecting the required blood to anticoagulant ratio, as under-filling of tubes leads to a high final concentration of K_3EDTA, which affects values of HCT, MCV and MCHC and thus interpretation of haematological results in dogs.

Key words: anticoagulants; edetic acid - pharmacology; hematologic tests; blood cell count; leukocyte count; dogs

Introduction

Several studies have revealed that various anticoagulants used for collection of blood specimens produce different effects on results of haematological analyses (1, 2, 3, 4, 5). Ethylenediaminotetraacetic acid (EDTA) is the anticoagulant recommended for complete blood cell counts (CBC) and white blood cell differential count (WCDC) by the National Committee for Clinical Laboratory Standards (6), principally for its cell preservation properties. Among sodium (Na) and potassium (K) salts of EDTA, the International Council for Standardization in Hematology (ICSH) currently recommends the dipotassium salts of EDTA (K_2-EDTA) as the anticoagulant for CBC (7). Under optimal conditions (appropriate anticoagulant concentration and analysis within 1-4 h of sample collection), the choice of K_2 or K_3-EDTA has little effect on the results of CBC and WCDC (8).

However, EDTA, even when using recommended EDTA concentrations, is known to have deleterious effects on platelets (9, 10, 11, 12, 13), erythrocytes (1, 13, 14, 15, 16) and leukocyte counts (5, 17, 18, 19) and occasionally dogs and cats have strong aggregations of platelets or white blood cells (WBCs) in EDTA blood tubes (20, 21, 22). The collection of blood in other anticoagulants (e.g., heparin, citrate) sometimes prevents or

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slows the aggregation allowing more accurate WBC counting (23).

The phenomenon of *in vitro* EDTA-induced platelet (PLT) clumping or agglutination is a well-recognized artefact, commonly reported in human medicine (10, 12, 15, 17, 18, 24, 25). The mechanism of agglutination or adherence (clumping) appears to involve EDTA-dependent platelet agglutinins (specific antibodies) present in the plasma (10, 26, 27, 28, 29, 30). There are few reports on EDTA-induced platelet clumping in veterinary medicine (31, 32, 33). EDTA-induced platelet clumping causes spuriously low PLT counts (pseudothrombocytopenia - PTP), which may lead to erroneous diagnosis, unnecessary and costly additional laboratory examinations, and inappropriate medical and surgical therapy or the unjustified withdrawal of essential medication. PTP may be accompanied by either spuriously high white blood cell (WBC) counts (pseudoleukocytosis) or occasionally spuriously low WBC counts (PLT-WBC adherence - pseudoleukopenia). Pseudoleukocytosis is due to the formation of PLT clumps large enough to mimic white cells on the WBC side of an impedance counters (Coulter S Plus IV/V, Technicon H*6000, Ortho ELT 8), whereas pseudoleukopenia may be due to the "gating out" of large PLT-WBC masses (5, 17, 18).

Blood sampling following a recommended blood to EDTA anticoagulant volume ratio is necessary to prevent coagulation. Under-filling is probably the most common phenomenon in clinical laboratories due to problems during blood sampling, particularly when blood is collected from very small, obese, severely hypotensive or restless patients. Under-filling of the tubes containing EDTA results in an excess of EDTA, which can reduce PCV and affect other haematological parameters (1, 2, 15, 23, 34).

The aim of the present study was to investigate the influence of high anticoagulant K_3EDTA concentration, produced by under-filling of tubes, on CBC and WCDC (white blood cell differential count) in healthy beagle dogs.

Material and methods

Animals

Thirty beagle dogs, eighteen females and twelve males, ranging from 8 months to 3 years, were selected and deemed healthy on the basis of history, results of physical examination and serum biochemical profiles (urea, creatinine, sodium, potassium, total bilirubin, albumin and alanine-aminotransferase - results not shown).

Sample collection

Venous blood samples were collected from fasted dogs using 5 mL syringes; 3 mL and 1 mL blood samples were put in two separate vacuum tubes containing K_3EDTA (5.40 mg) as anticoagulant, prepared for the collection of 3 ml blood. The final concentrations of K_3EDTA were: 1.8 mg/mL (recommended concentration) and 5.4 mg/mL (high concentration).

Both K_3EDTA blood samples for CBC and WCDC determination were stored at room temperature until analysis. Venous blood samples were also collected into plain tubes, which stood for 30 min at 4°C to clot, prior to centrifugation (1200 g for 10 min) and separation of serum.

Determination of haematological parameters – CBC and WCDC

CBC and WCDC were determined by an automated laser haematology analyser Bayer - Technicon H*1 with species-specific software (H*1 Multi-Species V30 Software). The measurements were performed within 1-4 h after venipuncture (5). CBC included white blood cells (WBC), red blood cells (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT). WCDC represented a six-part differential (neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophils (EOS), basophils (BASO) and large unstained cells (LUC). The LUC category consists of a heterogeneous population of all large cells that fail to exhibit any peroxidase activity (atypical lymphocytes, immature granulocytes and blasts).

Blood smears were made from single drops of blood anticoagulated with EDTA. Staining with May Grunwald-Giemsa solution was followed by microscopic examination of the smears for the presence of platelet clumps.

Statistical evaluation

Statistical analyses were done by use of the statistical programme SPSS 10.0 for Windows (SPSS – statistical package for social sciences). Means and standard deviations were calculated for all haematological and biochemical parameters. A paired *t* test was used to determine differ-

ences in CBC and WCDC parameters between blood samples with recommended and high K_3EDTA concentration. A value of $P<0.05$ was considered significant.

Results

The results of haematological analyses showed the influence of high K_3EDTA concentration on the following parameters of CBC (HCT, MCV and MCHC), while the parameters of WCDC were not affected (Tables 1 and 2). There was a significant ($P<0.05$) decrease in HCT of 13.4 ± 4.8% (from 0.55 ± 0.043 to 0.48 ± 0.042) and MCV of 11.9 ± 4.2% (from 74.4 ± 1.1 to 65.4 ± 3.0 fL), and a significant increase in MCHC (from 306.5 ± 5.7 to 349.7 ±17.8 g/L) of 14.0 ± 5.9% in samples with high K_3EDTA concentration compared to samples with the recommended final concentration of K_3EDTA.

Platelet clumping was not detected in any of the blood smears. No significant differences in PLT count (Table 1) were found between samples with recommended and high K_3EDTA concentrations. Despite the significant differences in HCT, MCV and MCHC between samples with recommended and high K_3EDTA concentrations, the CBC and WCDC parameters (mean values) in both types of samples remained within the normal reference ranges given by the producer of the analyser and data from the literature (35, 36, 37).

Discussion

Despite appropriate sample collection techniques, under-filling of tubes with blood and platelet clumping remains a common problem in veterinary medicine (31, 38, 39). Under-filling of tubes containing anticoagulant EDTA results in higher concentration of EDTA, which is known to cause spurious interferences, even at the recommended concentration, on platelets (5, 9, 10, 11, 12), erythrocytes (1, 13, 14, 15, 16) and leukocyte counts (5, 17, 18, 19). Occasionally, dogs and cats have strong aggregations of platelet or white blood cells (WBCs) in EDTA blood tubes (20, 21, 22, 23).

The results of the present study in healthy beagle dogs showed no significant changes in MPV and PLT count between samples with recommended and high K_3EDTA concentration. Unlike the present study, significantly lower PLT count has been measured in bulls in samples with high K_3EDTA concentrations due to EDTA-induced platelet clumping observed in blood smears (33).

In the present study, a significant decrease in HCT and MCV and a significant increase in MCHC was found in under-filled samples with high K_3EDTA concentration compared to the recommended K_3EDTA concentration. The significant changes of HCT, MCV and MCHC are ascribed to shrinking of erythrocytes in a hypertonic medium. Our results are consistent with the study of Chen et al. (1) who investigated the influence of under-filling of EDTA tubes using different human blood collection volumes. All salts of EDTA are hyperosmolar, which causes water to leave the cells, resulting in cell shrinkage. The higher the concentration of EDTA, the greater the osmotic withdrawal of water from the cells, leading to a reduction in PCV. This discrepancy will lead to a reduction in MCV and increase in MCHC (34). When insufficient blood is collected, such as frequently happens in small, obese, hypotensive and restless dogs, and also in cats, accuracy of HCT, MCV and MCHC is affected if K_3EDTA is used as the anticoagulant.

Following the results of our study, we recommend respecting the required blood to anticoagulant ratio, as under-filling of tubes leads to a high final concentration of K_3EDTA, which affects values of HCT, MCV and MCHC and thus interpretation of haematological results in dogs.

Table 1: Influence of K_3-EDTA on CBC in dogs

	WBC ($x10^9$/L)	RBC ($x10^{12}$/L)	HGB (g/L)	HCT* (L/L)	MCV* (fl)	MCH (pg)	MCHC* (g/L)	PLT ($x10^9$/L)
1.8 mg/mL K_3EDTA	9.04± 1.55	7.38± 0.60	168.1± 13.1	0.55 ± 0.043	74.4 ± 1.1	22.8± 0.5	306.5 ± 5.7	299.4± 65.5
5.4 mg/mL K_3EDTA	9.48 ±1.71	7.27± 0.55	166.0± 11.9	0.48 ± 0.042	65.4 ± 3.0	22.8± 0.4	349.7± 17.8	271.8± 70.6

*P< 0.05, paired t-test

The comparison of CBC values (mean ± SD) in blood samples of 30 healthy dogs with recommended concentration (1.8 mg/mL) and high concentration (5.4 mg/mL) of anticoagulant K_3EDTA

Table 2: Influence of K_3-EDTA on CBC in dogs

	NEUT %	LYMP %	MONO %	EOS %	BASO %	LUC %
1.8 mg/mL K_3EDTA	59.09±7.03	32.50 ± 7.09	2.86 ± 1.19	4.78 ± 3.21	0.18 ± 0.06	0.57 ± 0.24
5.4 mg/mL K_3EDTA	58.21±6.83	33.33 ± 6.69	3.13 ± 1.54	4.52 ± 3.23	0.28 ± 0.07	0.52 ± 0.19

The comparison of WCDC values (mean ± SD) in blood samples of 30 healthy dogs with recommended concentration (1.8 mg/mL) and high concentration (5.4 mg/mL) of anticoagulant K_3EDTA. Statistical comparison using paired t-test did not indicate any significant difference

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VPLIV VISOKE KONCENTRACIJE ANTIKOAGULANTA K_3-EDTA NA KRVNO SLIKO IN DIFERENCIALNO BELO KRVNO SLIKO PRI ZDRAVIH BIGLIH

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Povzetek: Kalijeve in natrijeve soli EDTA (etilendiaminotetraocetna kislina) se kot antikoagulanti največ uporabljajo v rutinski hematologiji, kljub temu da negativno vplivajo na trombocite, eritrocite in levkocite že pri priporočeni koncentraciji (1,8 mg/mL krvi). Pogosto se zgodi, da zaradi težav pri odvzemu krvi pri zelo majhnih, debelih, hipotenzivnih ali nemirnih psih in mačkah, odvzamemo premajhno količino vzorca krvi v epruvete z antikoagulantom K_3-EDTA, kar povzroči višjo koncetracijo EDTA, kot je priporočena. Namen naše raziskave je bil, ugotoviti vpliv visoke koncentracije K_3-EDTA na krvno sliko in diferencialno belo krvno sliko pri psih pasme bigel. V epruvete s K_3-EDTA smo odvzeli 3 mL (priporočena količina) in 1 mL (premajna količina) krvi in s tem dobili priporočeno (1.8 mg/mL) in visoko (5.4 mg/mL) koncentracijo K_3-EDTA. Krvno sliko in diferencialno belo krvno sliko smo določili z laserskim hematološkim analizatorjem, ki je zasnovan na računalniškem programu, s pomočjo katerega lahko določamo krvno sliko in diferencialno belo krvno sliko ljudem in živalim različnih vrst. Z analizo krvnih razmazov smo ugotavljali morebitno prisotnost skupkov trombocitov. V vzorcih z visoko koncentracijo K_3-EDTA smo, v primerjavi z vzorci s priporočeno koncentracijo K_3-EDTA, ugotovili statistično značilno (P 0,05) znižanje vrednosti hematokrita z 0.55 ± 0.043 na 0.48 ± 0.042 (13.4 ± 4.8% znižanje) in MCV s 74.4 ± 1.1 na 65.4 ± 3.0 fL (11.9 ± 4.2% znižanje) ter statistično značilno zvišanje MCHC s 306.5 ± 5.7 na 349.7 ± 17.8 g/L (14.0 ± 5.9% zvišanje). Analiza krvnih razmazov ni pokazala prisotnosti skupkov trombocitov v vseh analiziranih vzorcih. Na osnovi rezultatov raziskave priporočamo, da se pri odvzemu krvi upošteva zahtevano razmerje med količino odvzete krvi in količino antikoagulanta, saj visoka končna koncentracija K_3-EDTA, ki je posledica premajhne količine odvzetega vzorca krvi, vpliva na vrednost hematokrita, MCV in MCHC ter s tem na interpretacijo hematoloških rezultatov pri psih.

Ključne besede: antikoagulanti; edetska kislina - farmakologija; hematološki testi; krvnička, štetje; levkociti, štetje; psi