

MONITORING OF HEAMATOLOGICAL INDICES IN A SAMPLE GROUP OF CATS SUBJECTED TO SERIAL BLOOD COLLECTIONS FOR BIOEQUIVALENCE TESTING

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Abstract

The bioequivalence evaluation of a drug is based on the bioavailability of its active molecule. Serial blood sampling might be limited in cats, due to their reduced blood volume and other morphophysiological characteristics that will be analyzed in this study. The bioequivalence testing of an antihelmintic product was conducted on 37 common breed cats, between 2 to 4 kg, fed with dry and canned food. The testing protocol consisted of two phases, 14 days apart. Eleven blood samples were collected at increasing time periods (0-24h). The total blood volume collected in each stage was evaluated based on the volemia, estimated at 7.5% from the total body weight, representing a maximum of 13 ml/kg and 2.2 ml for each sample. Along with the serial blood sampling, at the start and ending of the two phases, haematological evaluations (on EDTA) and biochemical profiles (on Li-Heparin) were performed. Additionally, morphological assessments were carried out on panoptic stained smears. No major alterations of the physiological parameters were recorded, except for a small decline in the erythrocytic parameters, associated with oscillations of the total white blood cell count and a tendency to monocytosis. The results of this study reveal the necessity to associate physiological parameters of the tested animals with the requirements of drugs bioequivalence testing protocols, in order to respect ethical and good practice standards while collecting multiple blood samples.

Keywords: bioequivalence, cats, hematology, volemia.

INTRODUCTION

The bioequivalence tests require actions that facilitate sample collection, processing analysis and the interpretation of the recorded data. Collecting appropriate samples, both qualitatively and quantitatively, is essential for the success of these tests, with regard to the professional ethics, the existing laws and the good practice and animal welfare requirements. The protocols for these tests include a series of stress factors generated by the serial

collection of blood samples used for determining pharmacokinetic parameters and monitoring the evolution of o the haematological and metabolic profile indices.

Bioequivalence testing is based on the comparative assessment of bioavailability of two or more formulae of the same active ingredient, administered in the same way (Yilmaz and Elmas, 2010; Qayyum, 2012).

The bioequivalence of a drug product is considered to be achieved when the proportion and rate of absorption don't differ significantly from the ones recorded in the reference product, administered in the same molar dose (Altintas and Yarsan, 2009; Posyniak et al., 2001; Sumano et al., 2001, Martinez et al., 2001).

Systemic bioavailability is determined by the absorption time of the active substance, being influenced by the nature of the active substance, dosing form and their interactions with the absorption environment, bioavailability measuring the proportion of active molecules that are available to exert their action (Mycek et al., 1997; Stroescu, 19997, Mungiu, 1995).

The present paper aims to analyze the haematological test used to monitor the health in a group of cats used in bioequivalence investigations and the sampling and processing techniques used in these tests.

MATERIALS AND METHODS

The research has been carried out on a group of healthy adult cats (n=37), European race, originating from rural areas; there were 15 females and 22 males. When creating the group of animals to be included in the study, the following inclusion/exclusion requirements were taken into account (Table 1).

Table 1. Inclusion and exclusion criteria of the animals for the experiment

Inclusion criteria	Exclusion criteria
Male or female	Hypersensitivity to the administered formula or to other drugs containing it
Age 1-5 years	Acute diseases expressed 14 days before given the product
Negative results in the clinical and laboratory tests	Pregnant women and lactating
Weight 1-5 kg	Treatments using barbiturics/phenotyazinic in the last 30 days
	Case history regarding the evolution of major diseases (cancer, hepatic, kidney diseases, etc.)

During the two phases of the experiment, the cats were housed in individual cages, at the same time having permanent access to food and waters; the main food used was granulated feed, supplemented with canned food. As the testing protocol required, a 7 days period preceded the actual tests, needed for accommodation to the experimental conditions. During this period, the animals were subjected to usual clinical, haematological and biochemical tests to confirm their health status. The clinical tests followed mainly the evaluation of vital signs, additional clinical investigations, focused on the different apparatus, haematology and metabolic profile.

In each case, the maximal volume of blood that could be collected was determined using the weight ratio method.

Following the test protocol, the randomizing scheme was set up for the animals used for testing. Blood sampling was carried out from the brachiocephalic vein in most cases, except for few cases when blood was collected from the jugular or saphenous vein.

Blood samples were collected, at the beginning and at the end of the test, using EDTA as anticoagulant, for haematological test, and Li-Heparin, for the biochemical tests; during the tests, 22 samples of blood were collected from each animal, in the two phases, in order to determine the plasma concentration, at the intervals presented in Table 2.

Table 2. Time schedule for the blood sampling of the cats from the experiment

Interval	Schedule of sampling
Day 1	(0.0) and 0.5; 1.0; 1.5; 2.0; 4.0; 8.0; 12.0
Day 2	24.0 and 36.0
Day 3	48.0

Immediately after sample collection, the blood samples were centrifuged at 2000 G to separate the plasma necessary in the bioequivalence study.

The haematological tests were carried out using the Abacus Junior Vet automatic analyzer and consisted of: total and differentiated leukocyte count (WBC), red blood count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular

haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC). Morphological tests were also carried out separately, using blood smears coloured using the Dia-Quick Panoptic method.

The metabolic profile tests were carried out using the VetScan VS2 – ABAXIS high-throughput analyzer, using Comprehensive Diagnostic Profile kits for dogs and cats; the main indices determined were: total protein (TP), albumins (ALB), alanine aminotransferase (ALT), amylase (AMY) and globulins (GLOB) concentrations.

The individual data were statistically analyzed using the GraphPad InStat 3.05 software; arithmetic mean and standard deviation were calculated.

RESULTS AND DISCUSSIONS

Following the standards used in bioequivalence testing protocols, the tests were carried out on healthy animals. The experiment started with an initial selection based on the results recorded in the clinical, haematological and biochemical tests; after this selection, a total number of 15 cats were excluded from the test, 7 based on clinical criteria and 8 based on the laboratory criteria. Regarding the clinical tests, two cats were excluded based on their low body weight, two cats being pregnant, two cats being hard to control and one cat suffered from severe coagulopathy. As concerns to the haematological results, 8 cats were eliminated, deviations being recorded in the erythrocyte, leukocyte or biochemical parameters (Table 3).

Table 3. Subjects excluded from the testing and the criteria on which they were eliminated (n=15)

Examination	Nr of cases	Observations
Clinical	2	Low body weight
	2	Pregnancy
	2	Aggressiveness
	1	Coagulopathy
Haematological	5	Deviations from the physiological eritrocitary ranges
	3	Deviations from the physiological leucocitary ranges
Biochemical	3	Deviations from the normal metabolic ranges

Most of the animals that passed the inclusion/exclusion requirements stated in the test protocol, represented the experimental group, composed of 22 cats. Regarding them, it could be noted that the results of the clinical haematological and biochemical tests revealed that their vital functions were in normal physiological limits. The criteria used to select the feline donors for transfusion were chosen in order to estimate the volemia/total collected blood correlation (Kohn and Weingart, 2006). These criteria include the following requirements for the ideal feline donor: friendly temper, body weight of 4.5 kg, a minimum hematocrit of 35%. It is known that 10-15% of the total blood volume can be safely collected, the mean volemia in cat being estimated at 60.1 ± 9 ml/kg. So, the maximum amount of blood that can be collected from a potential donor is estimated at 11-13 ml/kg, once in 3-4 weeks (Donahoe, 2012). It is widely accepted that the acute loss of over 30% of the blood volume can determine hypo-volemic shock, with possible fatal consequences (Donahoe, 2012; Kohn and Weingart, 2006).

By analyzing the main erythrocyte parameters, recorded at the beginning and at the end of the experimental period, it can be observed that they are in normal physiological limits, indicating a good health status, without any signs of anemia (Figure 1). These values were constant throughout the two phases of the experiment, indicating that the serial blood sampling did not affect the main erythrocyte parameters, with regard to the experimental protocol and the good practice requirements. A global view of the erythrocyte mass parameters in the tested animal indicate that, despite the appearances, the cat is a suited animal for this type of blood sampling and can be used without reserve in bioequivalence studies of drugs.

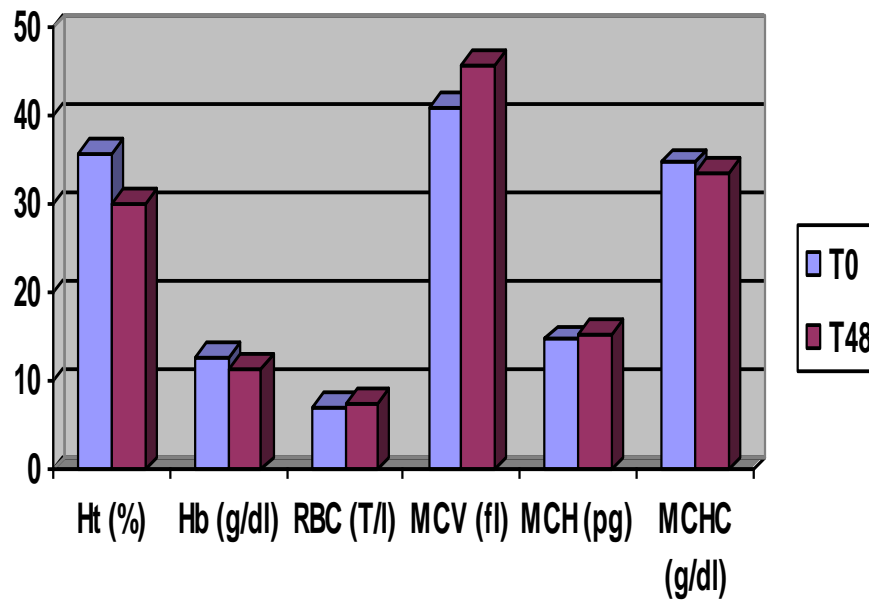
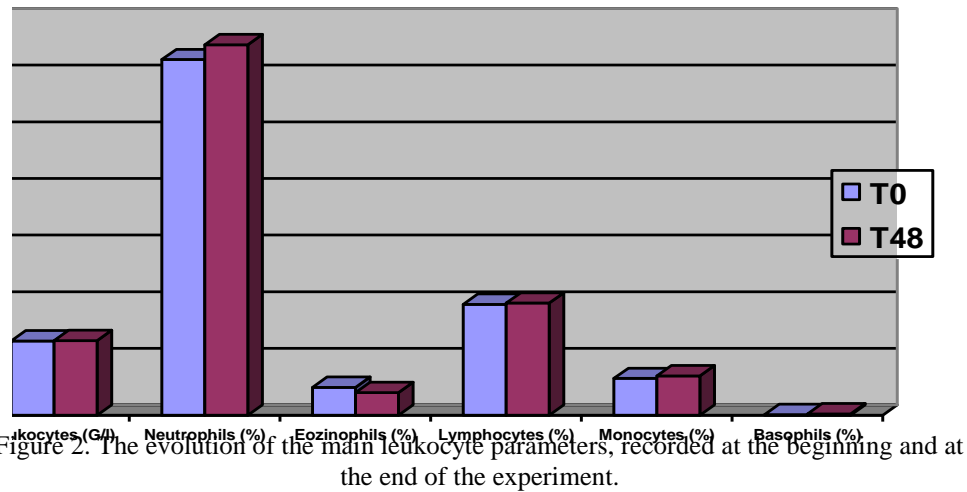


Figure 1. The evolution of eritrocitary parameters recorded at the beggining and end of the experiment

The evolution of the leukocyte parameters of the tested cats also revealed that serial sampling had no negative influence on the leucopoiesis, leukocyte functions and on the immune system. Special relevance, in this case, has the leukocyte parameters that maintained within the normal physiological range, throughout the experimental period (Figure 2).



The evolution of biochemical indices must also be analyzed from a general point of view. These parameters were determined at the beginning and at the end of the experimental period; as seen in table 4, these parameters were in the normal physiological ranges, cited by the literature (Campbell and Chapman, 2000).

The evolution of the metabolic profile indices also indicated that the serial blood sampling had no negative influence on the health status of the tested cats, also indicating that the animals adapted to the stress conditions generated by the bioequivalence tests (Figure 3).

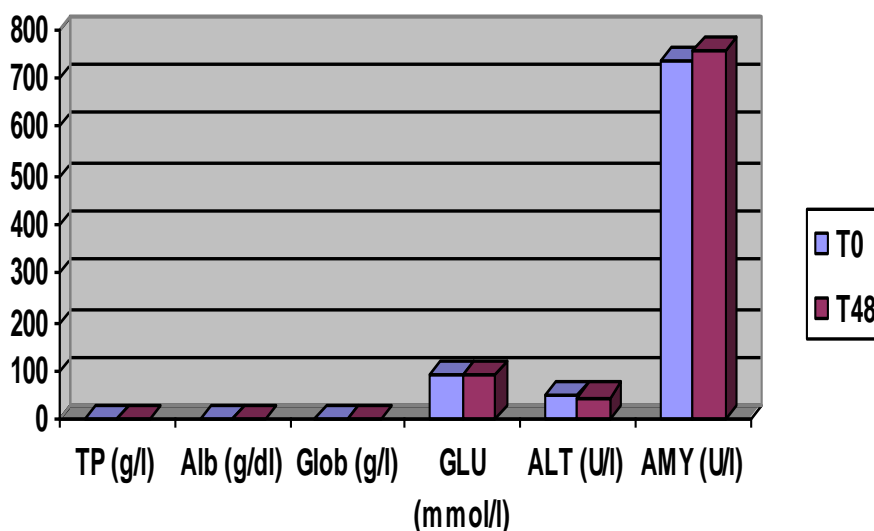


Figure 3. The evolution of the main biochemical blood indices, recorded at the beginning and at the end of the experiment.

The results recorded in this study must be correlated with the requirements of the European testing and approval rules and methods applied in the case of drugs for veterinary use. These imply the use of UE certified laboratory tests for monitoring health status of animals used in the pharmacokinetics and/or bioequivalence studies of various pharmaceutical formulae. Measuring the active substance concentration at the primary site of action, in biophase, is not yet possible, thus limiting the possibility to use these methods to assess the biological reaction or the therapeutic effect. These evaluations are based on determining the drug concentration in plasma or other body fluids; the intensity of the biological reaction is proportional to the plasma concentration of the substance determining that reaction (Posyniak et al., 2001).

The new testing methodology for drugs is focused on assessing and monitoring the risks and adverse effects that occur as a result of using various active molecules in veterinary therapy. All these aspects are reflected in quality and safety assurance of the drug, according to the European Union legal requirements.

CONCLUSIONS

Serial blood sampling allows determining the evolution of certain parameters within a timeframe; nevertheless they can act as stress factors.

All of the cats could be used for blood sampling from the brachiocephalic, saphenous or jugular vein, the last two being less used. The cannulas were well tolerated for 3 days, by the majority of cats; only two cases required changing the cannula during testing.

The total volume of blood collected during the serial blood samplings was within the limits given by the individual values of volemia (13 ml/kg), the initial and final analysis of the blood profile indicating non-significant decreases of the erythrocyte mass indices.

The leukocyte profile revealed small variations of the total leukocyte count, a mild monocytosis being also observed, confirming a good health status and the absence of adverse effects.

Comparative analysis of the biochemical indices revealed non-significant variations, with no consequences whatsoever on using cats in serial blood sampling based tests.

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