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NEPHROTOXICITY OF PREDNISONE IN CATS

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ABSTRACT

The nephrotoxicity of prednisone in cats was evaluated, using an immunosupressor dose (5 mg/kg/day), by establishing blood count, serum biochemistry (urea and creatinine) and urinary (GGT, protein and creatinine) parameters and urinalysis. Eight cats were used. The animals were of undefinied breed, adults and with average weight of 3 kg. They were housed in four cubicles and fed with two measures of commercial ration per day and water *ad libitum* during an adaptation period of seven days. The trial design was entirely random, being rejected any specific criterion of inclusion in this study besides the absence of any previous kidney damage. Animals received 5 mg / kg prednisone, orally, every 24 hours for a period of 14 days. The samples were collected in two different times, with the animals resting before prednisone administration (M1), and 14 days after the administration (M2). Significant differences were found in some hematological and biochemical parameters as well as in urinalysis. It was concluded that the prednisone (5 mg / kg / day), during a period of 14 days, is potentially nephrotoxic and the determination of the urinary GGT activity was proven to be precisely sensitive to early proximal tubular injury.

KEYWORDS: corticoid; feline; GGT; nephrotoxicity.

NEFROTOXICIDADE DA PREDNISONA EM FELINOS

RESUMO

Avaliou-se a nefrotoxicidade da prednisona em felinos com utilização de dose imunossupressora (5mg/kg/dia), por meio do hemograma, dos parâmetros bioquímicos séricos (uréia e creatinina) e urinários (GGT, proteína e creatinina) e da urinálise. Foram utilizados oito gatos, sem raça definida (SRD), adultos e com peso médio de 3 Kg. Os animais foram alojados em quatro baias (dois animais por baia) e alimentados com duas medidas de ração comercial por dia e água *ad libitum* por um período de adaptação de sete dias. O delineamento experimental foi inteiramente casualizado, sendo rejeitado qualquer critério específico de inclusão no trabalho, além da ausência de qualquer lesão renal prévia. Os animais receberam 5mg/kg de prednisona via oral a cada 24 horas por um período de 14 dias. As amostras foram coletadas em dois momentos, sendo animais em repouso antes da administração de prednisona (M1) e 14 dias (M2) após o início da administração do fármaco. Foram observadas diferenças significativas em alguns parâmetros hematológicos, bioquímicos e na urinálise. Conclui-se que a prednisona na dose imunossupressora (5mg/kg/dia) durante um período de 14 dias é potencialmente nefrotóxica e que a determinação da atividade da GGT urinária mostrou-se precisamente sensível à lesão tubular proximal precoce.

PALAVRAS-CHAVE: corticoide; felinos; GGT; nefrotoxicidade.

INTRODUCTION

The kidneys perform the function of eliminating final products of organic metabolism, and controlling the concentration of water and most constituents of body fluids. The kidneys perform their functions by the main mechanisms of glomerular filtration, tubular reabsorption and tubular secretion of various substances (MILLS et al., 1998).

The method routinely used to assess kidney function is the measurement of plasma concentrations of substances normally excreted by the kidneys. The assessments of urea and creatinine levels are the most common tests used. The evaluation of such data depends on the understanding of the extra-renal factors, which can affect them, besides the concomitant analysis of other laboratory data to find the cause of the increase in these substances. However, azotemia of renal origin only occurs when about 75% of the renal parenchyma is lost and, thus, the use of other methods that allow earlier identification of the renal pathology becomes very important for a better prognosis of renal failure (SANTIN et al., 2006).

The clinical enzymology is of great diagnostic importance (WESTHUYZEN et al., 2003) regarding the enzymes present in the blood stream and urine. Enzymology studies began in 1901 with Vitor Henri and have been intensified since 1910 by Leonor Michaelis. The first enzyme – alkaline phosphatase – was only described in 1927 by King and Armstrong. In the 1960s, enzymology began being used in the diagnosis in human medicine and in the 1980s its use was expanded for diagnosis in Veterinary Medicine (RODRIGUES, 2005).

Urinary enzymes are used for early detection of kidney injury. Among them, urinary gamma-glutamyl transpeptidase (GGT) is more easily tested (RAMBABU & PITTABIRAMAN, 1982; SODRÉ et al., 2007). Elevations in urinary enzyme activity occur even before changes in creatinine clearance, in serum creatinine concentration or in the electrolytes fraction excreted in the urine (MEYTS et al., 1988).

However, for an accurate determination of urinary GGT activity, it is necessary to carry out the correction calculation described by DESCHEPPER et al. (1989), using an urinary density of 1025 as correction factor for urinary flow of a single sample, such that $X = Y \cdot 25/Z$, where X is the urinary GGT activity calculated; Y is the urinary GGT activity of the sample, and Z corresponds to the last two digits of urinary density of the sample.

Gamma-glutamyl transpeptidase is a microsomal and membrane enzyme, of broad distribution in tissue involved in secretory and absorptive processes, particularly in the bile duct and the brush border of the kidney tubules (TASCI et al., 2005). It consists of a heavy (62-68Kda) and a light subunit (22Kda) (YU et al., 2007), and is abundantly present in the pancreas, liver, spleen, heart, brain, seminal vesicles and kidneys (TATE & ROSS, 1977; FRIELLE & CURTHOYS, 1982; CABRERA-ABREU & GREEN, 2002; ELIAS et al., 2004), besides being essential in maintaining the homeostatic balance, relative to oxidative stress (YU et al., 2007).

Urinary GGT reflects the lesion in the brush border of proximal tubules (KANEKO et al., 2008; SANTIN et al., 2006) and may be found in urine in harmful conditions, before other elements (RAMBABU & PATTABIRAMAN, 1982), besides persisting for a longer time (Yu et al. 2007).

The effects of steroids may be observed in almost any organism, because they affect the function of most cells (PENILDON, 2000). According to ANDRADE (2002), the steroid has a role in the metabolism of arachidonic acid by inhibiting the action of key enzymes, such as phospholipase A-2 and cyclooxygenase, the latter having tonic gene expression.

The cyclooxygenase are essential enzymes for the synthesis of prostaglandins from the release of arachidonic acid (AA) from the cell membrane by phospholipases. The oxidation and the subsequent AA reduction are responsible for producing, respectively, endoperoxidase (PGG_2) and hydroxyl endoperoxidase (PGH_2). The PGH_2 is converted via enzymatic and non-enzymatic mechanisms into primary prostanoids, prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin D_2 (PGD_2), thromboxane B_2 (TXB_2), Thromboxane A_2 (TXA_2) and prostacyclin (PENILDON, 2000).

Prostanoids synthesis, which involves the use of two oxygen molecules (MURRAY et al., 2002), occurs gradually, by means of a microsomal enzymes complex, and the prostaglandin G / H endoperoxide synthase (cyclooxygenase), in two distinct isoforms, is the first enzyme of this synthetic route. The cyclooxygenase 1 (COX-1) is constitutively expressed in most cells, on the other hand, COX-2 has enhanced its regulation by cytokines, shear forces and growth factors. COX-2 is the primary source of prostanoids formed in inflammation and cancer (BRUNTON et al., 2007).

According to PENILDON (2000), it is likely that COX-2 carries a role in renal physiology and

nerve conduction, so that inhibition of this enzyme may lead to organ dysfunction.

The aim of this study is to evaluate the nephrotoxicity of prednisone in cats with use of immunosuppressive doses (5mg/kg/day), by determining the urinary GGT activity and protein / creatinine relation in urine.

MATERIAL AND METHODS

This study was approved by the Ethics and Biosafety Committee of Federal University of Espírito Santo - UFES, under the protocol number 010/2008.

We used eight adult, mongrel cats, with an average weight of 3 kg. The animals were housed in four pens (two animals per pen) and fed two amounts of commercial feed[1] per day and water *ad libitum* for an adjustment period of seven days. After the acclimation, we collected blood samples for hemogram, biochemical parameters determination (urea and creatinine) and urine samples (urinalysis, protein / creatinine and GGT) in order to establish the arithmetic mean as control value.

The experimental design was randomized, and any specific criteria for inclusion in the study, besides the absence of any prior renal injury, was rejected.

The animals received 5mg/kg of oral prednisone every 24 hours for a period of 14 days. We collected samples, in the morning, in two different phases: When animals were at rest before prednisone administration (M1); and 14 days after the beginning of prednisone administration (M2). We obtained blood samples for blood count and biochemical parameters determination through puncture of the jugular vein, and we placed the samples in a tube containing EDTA at 10% and in another one without anticoagulant, respectively. We obtained the urine sample by cystocentesis.

We performed the urinalysis, primarily by observing the physical aspects (size, color, appearance and density), then the biochemical examination, using tapes[2] for biochemical analysis in order to determine the levels of glucose, ketone bodies, urobilinogen, protein, pH, occult blood and bilirubin. After this evaluation, we centrifuged urine samples in test tubes at 2000 revolutions per minute (RPM), discarded the supernatant, leaving a small fraction for homogenization and resuspension of the pellet. Soon after, we put up a sample on a slide and under a coverslip for further analysis under an optical microscope.

For the determination of serum urea, creatinine and urinary protein and creatinine and GGT, we used Commercial reagent kits[3], used in semi-automatic devices[4]. We carried out the hemogram according to the description by THRALL (2007); however, we performed the cell count with the aid of an automatic cell counter[5] and an optical microscope.

The values of urinary GGT activity were corrected by the formula described by DESCHEPPER et al. (1989).

RESULTS AND DISCUSSION

Laboratory findings regarding the number of erythrocytes, globular volume and total and differential leukocytes remained within the expected during a steroid therapy at a dose of 5mg/kg/day (immunosuppression dose).

We used the reference values adopted by JAIN (1993) for the evaluation of hemogram data. Mean values and standard deviations of the hematological parameters evaluated are shown in Table 1.

Statistical analysis of the mean values of red blood cells, hemoglobin, globular volume (GV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelet count showed levels within the standard variations for healthy animals in both collections, with a slight increase of GV after 14 days of prednisone administration. These data are supported by PEREIRA et al. (2007), who proved the existence of only small effects of steroids on erythropoiesis and hemoglobin concentration, and by FELDMAN & NELSON (1996), who found, in a study with 34 cats with hyperadrenocorticism, levels of erythrocytes and leukocytes consistently within the normality limits.

The results we obtained with the total leucocyte count showed values within the normal range at both collection times. However, the comparison of the evolution between the initial and the final moment showed significant decreases of mean eosinophils, monocytes and lymphocytes, as stated by PENILDON (2000) and PEREIRA et al. (2007). Segmented leukocytes showed no statistically relevant alterations, contrary to findings by DAMIANI et al. (1984), DAMIANI et al. (2001) and LONGUI (2007) regarding the effect of glucocorticoids in reducing apoptosis and the

[1] Cat chow® - Purina
[2] Combur Check - ROCHE®
[3] Kits Bioclin®
[4] BIOPLUS® BIO-200
[5] CELM® DA-550

increase in survival rate of neutrophils. We did not find basophils and rods at any moment.

For biochemical analysis, we used the normal values proposed by KANEKO et al. (2008). Mean values and standard deviations of the biochemical parameters are shown in Table 2.

TABLE 1. Means, standard deviations (SD), minimum and maximum values of hematological parameters (M1: control; M2: after 14 days) in animals treated with 5mg/kg of prednisone

Parameter	Period	Average	SD	Maximum	Minimum
RBC (x10^6/µL)	M1	7.67	1.44	10.21	6.22
	M2	7.33	1.19	9.02	5.71
Hemoglobin (mg/dL)	M1	12.6	3.52	14.6	9.8
	M2	12.9	3.32	15.2	8.4
Globular volume (%)	M1	36	4.54	42	31
	M2	38	7.12	47	29
MVC (fL)	M1	47	4.00	53	41
	M2	53	2.00	56	50
MCHC (%)	M1	33	1.5	35	31
	M2	34	1.2	35	31
Leukocyte (/µL)	M1	19,300	9,300	36,700	10,000
	M2	16,583	8,017	24,600	10,600
Neutrophils (/µL)	M1	12,264	7,350	25,323	7,000
	M2	12,551	6,460	18,942	5,560
Lymphocytes (/µL)	M1	5,347	2,572	7,992	1,500
	M2	2,726	1,823	6,811	1,320
Monocytes (/µL)	M1	873	594	2,202	292
	M2	803	597	2,214	318
Eosinophils (/µL)	M1	816	328	1468	399
	M2	443	258	973	172
Platelets (/µL)	M1	300,000	50,000	380,000	240,000
	M2	280,000	55,000	360,000	220,000

RBC: red blood cells; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration.

TABLE 2. Means, standard deviations (SD), minimum and maximum values of biochemical parameters (M1: control; M2: after 14 days) in animals treated with 5mg/kg of prednisone

Parameter	Period	Average	SD	Maximum	Minimum
Urea (mg/dL)	M1	48.8	8.2	57.5	34.6
	M2	36.8	16.8	61.6	11.8
Creatinine (mg/dL)	M1	1.31	0.23	1.70	0.90
	M2	1.03	0.26	1.60	0.80
Urinary GGT (UI/L)	M1	22.96	14.47	47.20	8.30
	M2	296.83	449.26	1259.00	32.90

None of the animals showed azotemia and / or uremia during the period of use of prednisone. The values we obtained for serum creatinine and urea did not present any statistically significant alteration able to develop azotemia. However, one cannot rule out the possible presence of renal lesions (BARBER, 1996).

The results of the analysis of the protein / creatinine relation performed at M1 were within normal limits. In comparison, the protein / creatinine relation at M2 was altered (three times higher than the standard deviation related to the average of M1), ensuring with 99.8% safety, a statistically significant change. These values indicate mild to moderate protein loss (BARBER, 1996). However, the results were compromised by the presence of different values of the means. Even so, the minimum values were above those determined in the normal range, which ensures the presence of kidney damage.

Therefore, the results of protein / creatinine relation we found in this work enable us to assume that the use of immunosuppressive dose of prednisone (5mg/kg/day) over a period of 14 days is potentially nephrotoxic for cats.

To analyze the results of urinary GGT activity, we used the mean obtained in the first test (M1) as a reference value. We noticed significant increase in urinary GGT activity in all animals after treatment; however, there was a significant variation among individuals with extremely discrepant values from the final mean. These findings are consistent with the ones by RIVERS et al. (1996) regarding the sensitivity of urinary GGT activity as a marker of renal injury.

The urinary density remained within normal levels (Table 3), which, according to OSBORNE & FINCO (1995), range between 1.035 and 1.065. However, the final values decreased slightly compared to M1, which can be justified by polyuria and polydipsia frequently observed in patients using steroids (ANDRADE, 2002).

TABLE 3. Means, standard deviations (SD), minimum and maximum values of urinary density (M1: control; M2: after 14 days) in animals treated with 5mg/kg of prednisone

Parameter	Period	Average	SD	Maximum	Minimum
Urinary density	M1	1.059	22	1.080	1.015
	M2	1.037	22	1.064	1.006

At control time, 87.5% of the animals did not present proteinuria. The other animals showed only traces of protein in urine. Significant proteinuria was detected in 37.5% of cats after 14 days of treatment. However, it is worth noting the variation among animals with protein loss and the clinically significant reversion of the situation in an individual.

WATERS et al. (1997) predicted proteinuria. The authors stated that protein loss is related to the administration of prednisone in dogs, suggesting that other animal, such as cats, can also present this alteration. However, FELDMAN & NELSON (1996) argued that urinary abnormalities associated with canine Cushing's syndrome (proteinuria, pyuria and bacteriuria) are not common in cats with this disease.

CONCLUSION

The determination of urinary GGT activity and the urinary protein / creatinine relation proved to be important as markers of renal function. Moreover, the determination of urinary GGT activity proved to be sensitive to early proximal tubular lesion; however, it was not effective to estimate the degree of renal injury. Overall, the urinary GGT showed to be sensitive as early marker of renal tubular injury experimentally induced in cats by prednisone.

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