THE EFFECT OF GENERAL ANESTHESIA ON GLOMERULAR FUNCTION AND KIDNEY TUBULAR INJURY IN DOGS

(Efeito da anestesia geral na função glomerular e injúria tubular renal em cães)

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ABSTRACT

The aim of this study was to investigate the possible occurrence of changes in renal function and tubular damage in dogs that underwent general anesthesia for elective orchiectomy or ovariohysterectomy. Thirteen dogs were selected, five males and eight females, with ages ranging from one to eight years, treated at the Polyclinic Veterinary School, Rio de Janeiro. Urine and blood samples were obtained from the animals just before and 30 minutes after the surgical procedure. Urinalysis (physical and chemical examinations and sedimentoscopy) was performed. The following biochemistries parameters were determined: plasma concentrations of urea and creatinine; urinary protein; urinary protein-to-creatinine ratio; and urinary GGT activity. A statistical difference was observed between the mean GGT activity before (0.38±0.21 U/L) and after (0.49±0.31 U/L) surgery ($p<0.05$). No changes in plasma creatinine and urea levels and other urinalysis parameters were observed. Thus, we conclude that the hypotensive effect of general anesthesia caused mild enzymuria, denoting possible renal tubular damage due to transient ischemia. This finding highlights the sensitivity and importance of studies of urinary GGT as an early marker of changes in renal tubular cells due to tubular injury.

Keywords: *Urinary gamma-glutamyltransferase, enzymuria, kidney function, dog.*

RESUMO

O objetivo deste estudo foi investigar a possível ocorrência de alterações na função renal e dano tubular em cães submetidos à anestesia geral para orquiectomia ou ovariohisterectomia eletivas. Treze cães foram selecionados, cinco machos e oito fêmeas, com idades variando entre um a oito anos, atendidos na Policlínica Veterinária Escola Rio de Janeiro. Amostras de urina e sangue foram obtidas dos animais antes e após 30 minutos do procedimento cirúrgico. Urinálise (exames físico, químico e sedimentoscopia) foram realizados. Os seguintes parâmetros bioquímicos foram determinados: concentração plasmática de ureia e creatinina; proteína urinária; relação proteína: creatinina urinária e atividade de GGT urinária. Diferença estatística foi observada entre as médias da atividade de GGT antes (0.38±0.21 U/L) e após (0.49±0.31 U/L) a cirurgia ($p<0.05$). Não foram observadas alterações nos níveis de ureia e creatinina plasmáticas e outros parâmetros da urinálise. Assim, foi possível concluir que o efeito hipotensor da anestesia geral causou discreta enzimúria, denotando possível lesão tubular em função da isquemia transitória. Estes achados destacam a sensibilidade e a importância de estudos da GGT urinária como um marcador precoce de alterações nas células tubulares renais em função de uma injúria tubular.

Palavras-chave: Gama-glutamiltransferase urinária, enzimúria, função renal, canina.

INTRODUCTION

Hemodynamic alterations, such as those induced by anesthetic drugs, can impair the renal blood perfusion, leading to localized hypoxia, causing lesions to the kidney tubular cells (HOLT, 2019). Multiple factors are involved in the pathophysiology of acute kidney injury in dogs. The most common etiologies include ischemia (RIMER *et al*., 2022) and all anesthetic

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agents can reduce the glomerular filtration rate (GFR), diminishing the renal blood flow as a consequence of systemic hypotension (NOEL-MORGAN and MUIR, 2018), due to renal vasoconstriction or interference in renal function via their cardiovascular effects, resulting in hypoxia. This in turn is an important cause of tubular damage, thus compromising the tubular integrity and functions (FUSELIER *et al*., 2007).

Although well-defined in human medicine, there is very little information regarding the association between general anaesthesia and acute kidney injury in animals (ROGERS-SMITH *et al*., 2020). Determination of urinary gamma-glutamyl transferase (GGT) can be used for diagnosis of early and persistent tubular injuries, which are associated with tubular necrosis (ILCHYSHYN *et al*., 2019; NIVY *et al*., 2021). The detection of enzymuria is specific for kidney tubular lesions because its urinary activity indicates renal origin, in turn since serum GGT is not filtered by the glomerulus due to its high molecular weight (ILCHYSHYN *et al*., 2019). Elevation in GGT-to-creatinine ratio has been demonstrated in experimentally induced nephrotoxicity due to gentamicin administration in the dog, suggesting the potential, clinical, diagnostic utility of urine GGT-to-creatinine ratio (RIVERS *et al*., 1996).

The aim of this study was to do the first report, in a pilot study, of the investigation of the possible occurrence of alterations in the serum and urinary biochemical parameters of dogs submitted to anesthesia for elective surgery.

MATERIAL AND METHODS

Location of the study and ethics aspects

This pilot study was conducted in the Multidisciplinary Laboratory of the Polyclinic Veterinary School of Valença University Center (UNIFAA) and it was approved by the Committee on Ethical Use of Animals (CEUA) of Valença University Center (UNIFAA) (protocol nº 40/2019).

Formation of experimental groups

Thirteen healthy dogs (five males and eight females, aged between 1 and 8 years), as determined by clinical and laboratory examination, were selected, without a history of previous kidney disease, among those brought by their owners to the School for elective orchiectomy or ovariohysterectomy. Two samples of blood (puncture of the cephalic vein) and urine (bladder catheterization or cystocentesis) were obtained from the animals just before and 30 minutes after the surgery. The time after the surgery was stablished based on early formation of urine after the anesthesia. If it was used too long afterwards, it is likely that urinary GGT could not be detected properly, since the enzymuria is right at the time of the injury.

Laboratory analyzes

To carry out the biochemical analyses (serum creatinine and urea, urinary protein and creatinine and urinary GGT), we used a BTS semiautomatic analyzer (Biosystems®, Brazil). The biochemical test kits used were of the same brand. The urinary GGT result was

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standardized in function of the urinary creatinine concentration (urinary GGT/urinary creatinine). A portion of each urine sample was reserved for testing of abnormal sediment elements (ASE), as well as physical and chemical exams (Combur Test® reagent strips, Brazil) and sedimentoscopy (optical microscope with 400x magnification).

The anesthetic protocol was the same for both males and females. Preanesthesia: acepromazine (0.05mg/kg, IM), morphine (0.4mg/kg, IM) and ketamine (0.5mg/kg, IM); induction: fentanyl (5mg/kg, IV) and propofol (5mg/kg, IV); maintenance: isoflurane (1% minimum alveolar concentration), a concentration that was varied slightly according to each animal.

Statistical analysis

The statistical analysis was performed using the software IBM SPSS® Statistics 22.0. The distribution of the data was analyzed by the Shapiro-Wilk test. Data with parametric distribution were submitted to the paired t-test, while data with nonparametric distribution were submitted to the paired Wilcoxon test. The level of significance in all tests was $p<0.05$.

RESULTS AND DISCUSSION

The results described below are expressed as mean ± standard deviation and reflect the parameters routinely used for evaluation of glomerular function and lesion as well as kidney tubular injury (ILCHYSHYN *et al*., 2019; MEUTEN, 2020). There was a statistically significant difference between the average GGT activity levels before (0.38±0.21 U/L) and after (0.49±0.31 U/L) surgery ($p<0.05$) (Fig. 01a), with increased enzymatic activity after surgery, representing a mild tubular lesion, possibly induced by a local hypotensive effect (HOLT, 2019; NIVY *et al*., 2021). Acepromazine, by blocking the actions that occur in the periphery by catecholamines, leads to hypotension secondary to vasodilation. Even with cases of arterial hypotension, the glomerular filtration rate (GFR) was maintained, which we can associate with a renal protective effect caused by the use of this drug (BOSTRÖM *et al*., 2003).

Previous studies have shown that urinary GGT is a sensitive early marker of abnormal serum creatinine levels for evaluation of kidney tubular lesions (MELCHERT *et al*., 2007; ILCHYSHYN *et al*., 2019), and GGT levels can also be altered due to ischemic injury (HOLT, 2019). Lastly, their high molecular weight precludes their glomerular filtration in absence of glomerular injury, and their presence in the urine is primarily of renal proximal tubular origin (NIVY *et al*., 2021). However, in the present study, under such conditions, considering the brief duration of the surgical procedure (average of 25 minutes for females and 15 minutes for males), associated with the slight variation in urinary enzymatic activity, led to the conclusion that the tubular lesions were only mild and it was not enough to drastically exceed the reference limits proposed in previous research carried out by other authors with similar methodologies.

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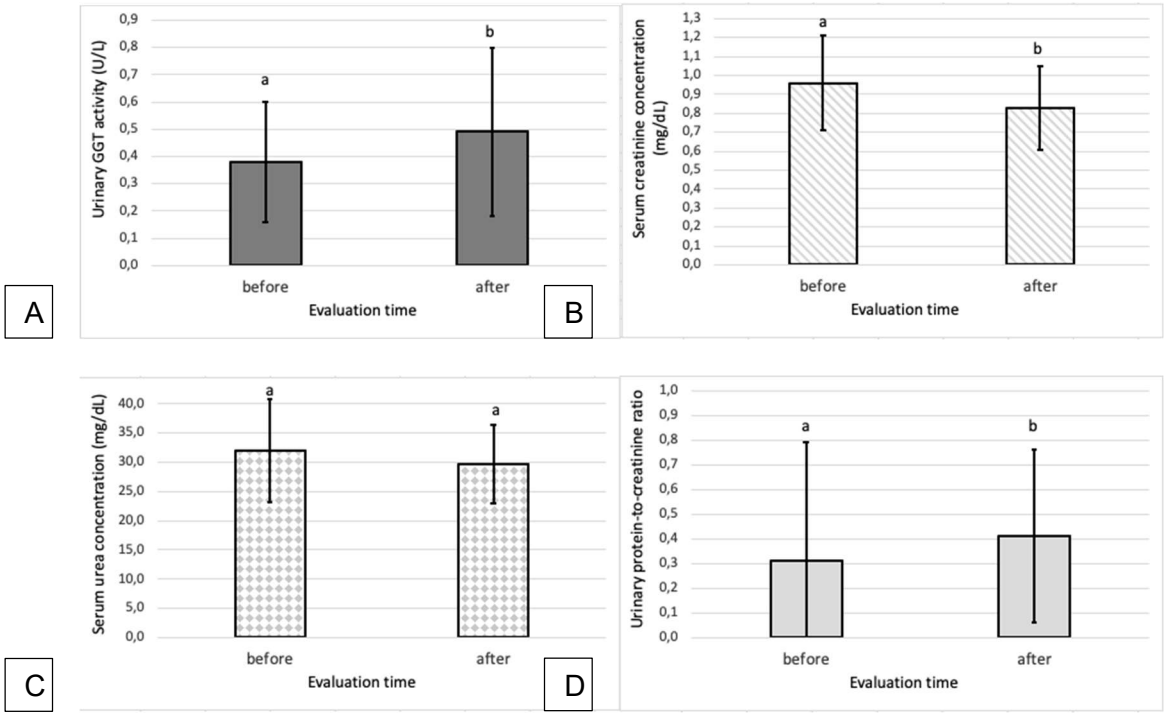

Figure 01: Comparison of serum and urinary biochemical parameters of dogs before and after the elective surgical procedure.

Obs.: A = Urinary GGT activity (U/L); B = serum creatinine concentration; C = serum urea concentration; D = urinary protein-to-creatinine ratio in dogs submitted to anesthesia for elective castration surgery. [a,a]Equal letters: the means do not differ significantly; [a,b]Different letters: the means are statistically different.

Even with a short duration of the procedure, these animals were submitted to general anesthesia to remain immobile during the procedure. (ILCHYSHYN *et al*., 2019; NIVY *et al*., 2021). It is worth mentioning that, as it is an unusual parameter that suffers strong methodological influences on its results, the use of a reference range adjusted to each method is necessary and the established references only direct to approximate results, not having good sensitivity for detect more subtle variations (MEUTEN, 2020). Thus, the comparison between the time before and after in the same animal allows a better observation of the effect of anesthesia on the tubular lesion. Further studies using more animals and other surgical procedures could be evaluated to better characterize this influence on enzymuria.

Fusellier *et al*. (2007) and Cattai *et al*. (2018) reported that the reduction of peripheral vascular resistance related to the use of propofol can lead to reduced systemic arterial pressure (SAP), causing a reduction of the renal blood flow. In the present study, the SAP was measured on average every 4 minutes during the operation and did not present any significant differences ($p>0.05$) between the evaluation times. Although the SAP level did not directly reflect alterations in renal blood flow, a more direct relationship can be established in case of more significant variations in the SAP values (CATTAI *et al*., 2018; NOEL-MORGAN and MUIR, 2018).

Serum creatinine concentration has been shown to be a predictor of mortality in many different disease processes in dogs (HARISON, 2012). In the comparison of the serum concentration of creatinine, there was a statistically significant difference ($p<0.05$), with postoperative decline of 14.5% on average (before: 0.97±0.26mg/dL; after: 0.83±0.22mg/dL)

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(Fig. 01b). The creatinine concentration did not exceed the minimum reference limit used in this study (0.5mg/dL). We attributed the difference observed to two possible causes: the effect of intraoperative dilution of fluid therapy and/or the relationship between glomerular filtration and renal blood flow, which can be maintained despite the presence of arterial hypotension, mainly due to having acepromazine as a pre-anesthetic medication, which ends up having protective effects on the rate glomerular filtration (BOSTRÖM *et al*., 2003).

This would explain the reduction in the serum creatinine concentration observed, since creatinine is an analyte that does not undergo tubular reabsorption (MEUTEN, 2020). Menezes *et al*. (2010), in an experiment involving induction of renal ischemia and reperfusion observed an increase of the average serum creatinine level beyond the normal pattern in dogs, reinforcing the low sensitivity of the parameter to this occurrence (DAVIS *et al*., 2021). The serum urea concentration was the only parameter that did not present a statistically significant difference in the comparison of the means (Fig. 01c), demonstrating that just as for creatinine, this parameter also has low sensitivity for evaluation of early alterations in kidney function (STOCKHAM and SCOTT, 2011). Similar results in urea and creatinine concentrations were also observed by Santos *et al*. (2018) using similar anesthetic protocol.

The urinary protein-to-creatinine ratio (UPCR) presented a statistical difference between the averages before (0.31±0.48) and after (0.41±0.35) surgery ($p<0.05$) (Fig.1d). However, in 46.1% of the animals we observed active sediment in the urinalysis after surgery, indicating an association of the increase in UPCR with post-glomerular proteinuria (STOCKHAM and SCOTT, 2011). That observation reveals the limitations of using this parameter as a specific marker of glomerular injury, since any post-renal cause should initially be discarded to enable attributing proteinuria to glomerular lesion. Although proteinuria might also be the result of primary glomerulardisease, a UPCR >2 alongside hypoalbuminemia is considered consistent with glomerulonephropathy (COLE *et al*., 2020).

Low-degree cylindruria (1+) was observed in five samples (one sample with hyaline cylinders and four with granular cylinders) in tests before and after. In all the dogs, the urine had density values greater than 1.040, which although not reflecting pathological alteration, is a possible finding in normal hypersthenuric urine (MEUTEN, 2020). Two of these samples came from the same animal, evaluated before and after surgery, with urine density values of 1.058 and 1.060, respectively. The other sedimentoscopy findings were not significant, and in all cases the quantities of transitional and epithelial cells and also presence of hematuria were low to moderate, mainly associated with the urine collection method. Vesical catheterization was used in the majority of cases. Besides the increase in the urinary GGT activity, Menezes *et al*. (2010) also reported the presence of granular cylinders, an alteration that in the present study could not be associated with the anesthesia since they were not observed in isolation in postoperative samples. Since traditional markers of tubulopathy (eg, cylinduria and glucosuria) are insensitive, more sensitive urinary biomarkers of renal tubular cells can improve early recognition of tubulopathy, and potentially aid inpredicting AKI and initiating timely therapy (NIVY *et al*., 2021).

Since the work carried out was a preliminary study of the effects of general anesthesia analyzed from the biochemical point of view related to enzymuria, further studies using a longest anesthesic period, including histopathological analyzes could better characterize the occurrence of lesions under such conditions. No animals died during the research.

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CONCLUSIONS

The hypotensive effect of the general anesthesia provoked mild enzymuria, indicating possible renal tubular lesion in function of transient ischemia. That finding highlights the sensitivity and importance of studies of enzymuria as an early marker of alterations in renal tubular cells due to various causes. No alterations were observed in the concentrations of serum creatinine and urea and proteinuria due to the effect of general anesthesia.

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