# Nonsteroidal anti-inflammatory therapy. Changes on renal function of healthy dogs[1]

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**ABSTRACT**

**PURPOSE**: To evaluate the renal function in healthy dogs submitted to nonselective and preferential COX-2 nonsteroidal anti-inflammatory drug (NSAID) therapy.

**METHODS**: Twenty four healthy dogs were distributed into four groups (G) (n=6): ketoprofenG – treated with ketoprofen; nimesulideG – treated with nimesulid; meloxicanG - treated with meloxican; and etodolacG – treated with etodolaco. All the dogs received the NSAIDs for 10 days by oral route. Physical examination and renal function (urinalysis, urinary sodium and gamma-glutamyl transpeptidase (GGT), serum urea, creatinine, potassium and sodium, and endogenous creatinine clearance) were evaluated before, after five and ten days (T0, T5 and T10) of the treatment in all groups.

**RESULTS**: Changes were observed in urinalysis, with a significant increase in renal cells in the urine at T5 and T10 in nimesulideG. Significant reduction in urinary sodium in nimesulideG at T5 was observed. The clearance values were lower in ketoprofenG at T10.

**CONCLUSIONS**: Meloxicam and etodolac were the drugs that have proven to be safer for short-term therapy in healthy dogs in relation to renal function. NSAIDs ketoprofen and nimesulide should be used judiciously in dogs with renal dysfunction, since there are promoted changes in renal function.

**Key words**: Anti-Inflammatory Agents, Non-Steroidal. Drug Effects. Kidney. Dogs.

## Introduction

Nonsteroidal anti-inflamatory drugs (NSAIDs) are extensively used in humans and animals for pain relief and acute or chronic inflammatory conditions[1]. They are amongst the most common used therapeutic agents. There are more than fifty different NSAIDs on the market and there are still a large number of new preparations. The large number of new substances indicates that none act without deleterious effects[2].

The mechanism of action of NSAIDs is based on the inhibition of cyclooxygenases (COX), which promotes an inhibition of prostaglandins (PG) production, which is important in mediating pain and inflammation[1]. There are at least two types of COX which play different physiological functions in the body: COX-1 and COX-2[3].

Most NSAIDs are nonselective and inhibit both constitutive COX-1 and COX-2, while others may have greater or lesser degree of COX-2 inhibition. NSAIDs are classified in non-selective NSAIDs (ketoprofen, aspirin, naproxen, Flunixin meglumine, and others), preferential COX-2 inhibitors (e.g. meloxican, etodolac, nimesulide) and highly selective COX-2 inhibitors (coxibs)[1].

Most of NSAIDs collateral effects are attributed to COX-1 inhibition. NSAIDs that nonspecifically block COX, predisposes to collateral effects, especially in renal, gastroinestinal and circulatory tracts[3]. It is stated that COX-1 performs cellular "housekeeping" functions for normal physiologic activity and is the predominant isoform expressed in platelets and the GI tract. On the other hand, COX-2 acts at inflammatory sites causing the development of the COX-2 selective inhibitors, with the aim of providing similar efficacy and improved safety[4].

However, studies have shown that COX-1 is present in conjunction with COX-2 at sites of inflammation, while COX-2 can be expressed constitutively[4] in certain areas of the kidney, brain and endothelial cells for normal physiological functions[5]. Moreover, adverse effects were attributed to the more selective COX-2 inhibitors, such as high incidence of cardiovascular and thromboembolic events[6] and changes in the kidney functions in conditions such as dehydration, decreased renal perfusion or pre–existing renal damage[3].

Alterations in renal function are described amongst the several NSAIDs adverse effects[7]. These effects are strictly related to the reduction in prostaglandin (PG) synthesis induced by NSAIDs. The vasodilating effect of PGs plays a role in preservation of renal blood flow and glomerular filtration rate by relaxing the preglomerular resistance[8].

In Veterinary Medicine, particularly in small animals, there is a lack of therapeutic protocols that standardize the clinical use of COX-2 NSAIDs[9]. Therefore, the aim of this study was to evaluate the renal function of dogs submitted to the therapy with different non-selective and preferential COX-2 NSAIDs.

## Methods

The experimental protocols were approved by the Animal Research Ethics Committee of University of Oeste Paulista (UNOESTE), Brazil.

Twenty-four healthy adult mongrel dogs, females, with a body weight ranging from six to 15 kg were evaluated. Clinical and laboratorial evaluations were the inclusion criteria adopted to consider the healthiness of the animals[10-14]. The dogs received water and commercial feed *ad libitum*.

The dogs were randomly distributed into four groups (G) (n=6), that received for ten days, the following therapies with NSAIDs orally: ketoprofenG –ketoprofen (2 mg/kg, once daily); nimesulideG - nimesulide (5 mg/kg, once daily); meloxicanG - meloxican (0.2 mg/kg, on day 1, and 0.1 mg/Kg, once daily, until complete a protocol of 10 days of treatment); etodolacG – etodolac (15 mg/Kg, once daily)[1]. The NSAIDs were administered wrapped in canned food (Pedigree®), without fasting.

The physical examination, body weight and renal function (urinalysis, urinary sodium and gamma-glutamyl transpeptidase (GGT), serum urea, creatinine, potassium and sodium, and endogenous creatinine clearance) were evaluated before, after five and ten days of treatment (T0, T5 and T10, respectively). The general condition of animals and gastrointestinal tolerance (food intake, vomiting or diarrhea) to NSAIDs were assessed daily.

### *Renal function evaluation*

Urine samples were collected by urethral catheterization[15]. In the urinalysis, urine specific gravity was measured by refractometry (Atago Refractometer®). Urine pH and other chemical parameters were evaluated with a reagent test strips (Combur[10] Test UX® - Roche Diagnostics). The urinary sediment was performed after urine centrifugation and examined under a light microscope using the x40 objective. The urinary GGT enzyme was determined by kinetic UV method (Gamma GT Liquiform, Labtest, Brazil).

The endogenous creatinine clearance was performed in two subsequent periods of 20 minutes[15]. Bladder contents were removed every 20 minutes for measurement of urine volume and

determinations of urinary creatinine. Blood samples were collected at the midpoint time, for serum creatinine analysis. The clearance was determined by multiplying the urine flow rate (mL/min) by the creatinine (Cr) concentration in the urine, divided by serum creatinine, using the formula: GFR (mL/min) = urinary volume [mL/min] X urinary creatinine [mg/dL]/serum creatinine [mg/dL][16]. The result was divided by the animal's body weight (kg) in order to obtain the estimate of glomerular filtration rate (GFR) in mL/min/kg.

Blood samples were collected from the jugular vein for biochemical profiles. Urea was measured by an end-point urease method and creatinine by the alkaline picrate method (Labtest, Brazil). The serum sodium and potassium and urinary sodium were measured in electrolyte analyzer (AVL 9180 - Roche) and determined by the ion-selective method.

*Statistical analysis*

The data were expressed as mean ± standard deviation (SD). The data were compared using one-way analysis of variance (ANOVA), followed by the Dunnett's multiple comparison test in order to compare the differences in each treatment. Student-Newman-Keuls test was employed to evaluate differences among treatments at each time point. The differences were considered significant at $p<0.05$.

## Results

There were no changes in the physical examination in the dogs treated with different NSAIDs. At all time points evaluated, none of the animals showed evidence of renal pain in sensitivity test. Changes in general condition, body weight, food intake, vomiting or diarrhea were not observed.

The nimesulideG, urinalysis pointed out a significant increase in renal cells at T5 and T10 when compared to T0. The other parameters of urinalysis were within normal ranges for the canine species and revealed no differences between time points in each group or between different groups. The urinary enzyme GGT showed no variation between groups or time points. The urinary sodium excretion showed a significant increase in the ketoprofenG in T10 compared to T0. This finding has little clinical importance, since the animals received NSAIDs wrapped in canned food, rich in sodium. However, despite this fact, there was a significant reduction in sodium excretion in the nimesulideG at T5, when compared to T0 and T10 (Table 1).

**TABLE 1** - Urinary sodium (mEq/L) and endogenous creatinine clearance (mL/Kg/min) for ketoprofen, nimesulide, meloxican and etodolac groups.

| | T0 | T5 | T10 |
|---|---|---|---|
| **KetoprofenG** | | | |
| Urinary sodium | 82.0±14.8 | 112.0±31.4 | 130.7±41.5* |
| Clearance | 2.7±0.9 | 2.4±0.9 | 1.9±0.4 |
| **NimesulideG** | | | |
| Urinary sodium | 116.7±33.7 | 45.7±14.8† | 128.0±56.4 |
| Clearance | 2.7±0.7 | 2.9±1.0 | 2.7±0.7 |
| **MeloxicanG** | | | |
| Urinary sodium | 99.2±14.1 | 93.0±64.1 | 127.5±74 |
| Clearance | 2.8±1.0 | 2.7±0.7 | 3.0±0.6 |
| **EtodolacG** | | | |
| Urinary sodium | 84.5±44.2 | 111.3±40 | 90.3±62.1 |
| Clearance | 2.6±0.8 | 2.8±0.6 | 3.2±1.2 |

* $p < 0.05$ X T0; † $p < 0.05$ X T0 and $p < 0.01$ X T10.
Reference ranges: Urinary sodium = 96±38 (mmol/L) [7]; Endogenous creatinine clearance = 2.66 ± 0.14 (mL/min/Kg) [16].

There were no statistical differences among time points in each group or among different groups in the serum creatinine, sodium and potassium levels (Table 2), which remained within normal ranges at all time points evaluated. Regarding the urea, there was a significant increase in the concentration of this substance on T5 compared to T0 in etodolacG ($p <0.05$). However, the values of serum urea and creatinine remained within the normal range for dogs[11] at all time points in the other studied groups (Table 2). In the assessment of endogenous creatinine clearance, there were no statistical differences among different groups or among time points in each group. However, the ketoprofenG mean clearance values at T5 and T10 were below the normal range for dogs (Table 1).

**TABLE 2 -** Results of biochemical tests for ketoprofen, nimeulide, meloxican and etodolac groups.

| | Urea (mg/dL) | Creatinine (mg/dL) | Sodium (mEq/L) | Potassium (mEq/L) |
|---|---|---|---|---|
| **KetoprofenG** | | | | |
| T0 | 35.3±6.9 | 1.0±0.2 | 142.0±7.7 | 4.4±0.4 |
| T5 | 32.5±8.4 | 1.1±0.1 | 142.8±6.7 | 4.1±0.4 |
| T10 | 38.3±8.2 | 0.9±0.1 | 143.8±5.6 | 4.4±0.2 |
| **NimesulideG** | | | | |
| T0 | 41.5±9.2 | 1.0±0.1 | 144.3±4.7 | 4.5±0.3 |
| T5 | 42.4±9.8 | 1.0±0.1 | 142.2±3.5 | 4.3±0.4 |
| T10 | 47.1±11.9 | 1.0±0.1 | 143.0±5.4 | 4.4±0.4 |
| **MeloxicanG** | | | | |
| T0 | 36.5±6.6 | 1.0±0.1 | 144.0±3.0 | 4.4±0.3 |
| T5 | 40.9±11.6 | 1.1±0.1 | 145.8±3.5 | 4.4±0.7 |
| T10 | 41.7±11 | 1.1±0.2 | 143.0±3.3 | 4.1±0.2 |
| **EtodolacG** | | | | |
| T0 | 34.0±5.5 | 1.0±0.1 | 146.0±8.6 | 4.3±0.4 |
| T5 | 41.3±4.3* | 1.1±0.3 | 143.5±2.7 | 4.4±0.3 |
| T10 | 38.0±4.4 | 0.9±0.1 | 146.5±2.0 | 4.6±0.2 |

* ($p<0.05$) X T0
Reference ranges: Urea = 21-60 (mg/dL); Creatinine = 0.5-1.6 (mg/dL) [8]. Serum Sodium = 145-158 (mEq/L); Serum Potassium = 4.1-5.5 (mEq/L) [23].

## Discussion

The traditional NSAIDs act by inhibiting PG synthesis. It is assumed that the simultaneous inhibition of COX-2 mediates the antipyretic, analgesic and anti-inflammatory NSAIDs actions, whereas the simultaneous inhibition of COX-1 responds largely, but not exclusively, by adverse drug reactions[17], including mainly the gastrointestinal side effects[18].

In the kidney, prostaglandins play an important role in regulating the tone of blood vessels and salt and water balance. COX-1 and COX-2 are involved in renal blood flow regulation and tubular function[3]. In this study, even in the condition of dog healthiness, early changes (T5) in renal function were observed, resulting from the nimesulide treatment. Similar effects were observed in rats, despite therapy with nimesulide alone for five days did not produce any change in renal function, this aggravated the renal toxicity produced by cisplatin when used in combination[19]. Thus, based on the findings observed in this study, it is possible to corroborate the indication of nimesulide for a maximum of five days[1].

Renal and renovascular adverse events have been associated with the use of traditional NSAIDs (nonselective) and COX-2 selective[3]. The effects on renal blood flow may promote reversible, or even irreversible, renal failure (short therapies) in patients with nephropathy associated with high analgesics doses used continuously[16].

There is evidence that some non-selective NSAIDs have a lower nephrotoxic potential than the other NSAIDs[20]. The frequency of renal disease occurrence related to COX-2 inhibitors compared with nonselective NSAIDs is still unknown[2], which suggest the importance of studies that confront the evaluation of renal function under the regime of therapy with different NSAIDs, COX-2 selective or nonselective.

Sodium retention is not a rare side effect of NSAIDs, but is usually self-limited and minor. Since PGs are renal vasodilators and natriuretics, the mechanisms associated with NSAID use include decreased renal blood flow accompanied by increased tubular reabsorption of sodium[5], causing retention of salt and water[3].

The initial idea that COX-2 selective NSAIDs do not have adverse effects to the kidneys has been questioned. Clinical studies have shown that the functional role of intrarenal COX-2 is predominantly associated with the maintenance of electrolyte homeostasis[21], facts corroborated by this study, in which nimesulide, COX-2 preferential NSAID, was able to alter the excretion of sodium and urinalysis. In the nimesulideG, whose urinary excretion of sodium showed a decrease in T5, it would be expected an increasing in serum potassium levels. NSAIDs promote reabsorption of potassium as a result of reduced availability of sodium in distal tubules and the suppression of renin secretion induced by PGs[3].

NSAIDs have little effect on renal function and blood pressure in healthy humans. However, in the presence of heart failure, chronic kidney disease and hypovolemia, the formation of PGs is crucial in dogs[5]. These facts call attention to the importance of the choice of NSAID use in patients with diseases that predispose to hypertension, in which sodium retention can be deleterious. A generation of renal vasodilatory PGs was attributed to the COX-2 in mice. The researchers stated that the incidence of hypertensive complications induced by NSAIDs in patients may be correlated both with the degree and the selectivity of inhibition of COX-2 in kidney[6].

In this study, the only drug that produced a reduction in the renal elimination of sodium was the nimesulide. However, serum sodium levels remained unchanged in this group as well

as in the other groups. Although, it should be considered that the dogs evaluated in this study were considered healthy. Thus, use of nimesulide should be criteriously indicated in patients suffering from edematous conditions such as cirrhosis, chronic renal failure and congestive heart failure, which may cause the aggravation of edema[22].

For a better assessment of renal function, glomerular filtration rate (GFR) should be evaluated because it represents a good marker of progression of kidney disease or renal failure, even in early stages[23]. In the ketoprofenG, the lowest mean values of clearance were obtained at T T10 (1.9 ± 0.4), although significant differences between time points or groups were not found, confirming the assertion that the COX-1 is important in maintaining the function of GFR[21]]. The action of PGs generated by COX-1 dilates the vasculature, decreased renal vascular resistance and increases renal perfusion[5].

Renal toxicity associated with NSAIDs is characterized by decreasing in tubular function and renal perfusion, or by sodium and fluid retention. The decrease of PGs induced by NSAIDs can cause vasoconstriction with reduced renal blood flow and hence GFR. This mechanism may promote reversible or irreversible renal failure, depending on the susceptibility of the individual and the time of use of the drug[3].

In patients with glomerular disease, inhibition of PG synthesis by NSAIDs can lead to a reversible renal ischemia, a decline in glomerular hydrostatic pressure and ARF[20], since the increased production of PGs seems to keep GFR in the presence of a significant reduction in glomerular capillary permeability[8]. In this study, there were no significant changes in relation to renal markers. Nevertheless, minimal changes in renal function were observed in ketoprofen and nimesulide groups, in spite of the normal clinical conditions of the studied dogs and the short time of therapy. Thus, use of the NSAID ketoprofen and nimesulide in patients with pre-existing kidney disease must be indicated criteriously.

It is important to emphasize that an ideal molecule of NSAIDs that does not trigger a deleterious effect on the individual is not achieved. All NSAIDs have the potential to produce renal injury. This will depend on the individual susceptibility to the renal lesions[17]. Therefore, it is important to perform an accurate clinical evaluation of the renal function prior to the indiscriminate prescription of NSAIDs.

## Conclusion

Meloxicam and etodolac were the drugs that have proved safer for short-term therapy in healthy dogs in relation to renal function. NSAIDs ketoprofen and nimesulide should be used judiciously in dogs with renal dysfunction, since promoted changes in renal function.

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