RESEARCH PAPER

Cyclooxygenases 1 and 2 inhibition and analgesic efficacy of dipyrone at different doses or meloxicam in cats after ovariohysterectomy

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Abstract

Objective To evaluate the cyclooxygenases (COX) inhibition, adverse effects and analgesic efficacy of dipyrone or meloxicam in cats undergoing elective ovariohysterectomy.

Study design Prospective, blinded, randomized, clinical study.

Animals A total of 30 healthy young cats.

Methods The cats were randomly assigned to three postoperative groups: D25 (dipyrone 25 mg kg^{-1} every 24 hours), D12.5 (dipyrone 12.5 mg kg^{-1} every 12 hours) and M (meloxicam 0.1 mg kg^{-1} every 24 hours). In the first 24 hours, the drugs were administered intravenously (IV), and then orally for 6 (dipyrone) or 3 days (meloxicam). Prostanoids thromboxane B_2 and prostaglandin E_2 concentrations served as indicators of COX activity and, with physiological variables and pain and sedation scores, were measured for 24 hours after first analgesic administration. Rescue analgesia (tramadol, 2 mg kg^{-1} IV) was provided if Glasgow feline composite measure pain scale (CMPS-Feline) $\geq$5. Laboratory tests included symmetric dimethylarginine and adverse effects were evaluated regularly up to 7 and 10 days after surgery, respectively. Parametric and nonparametric data were analyzed with two-way ANOVA and Kruskal-Wallis tests, respectively ($p < 0.05$).

Results In the first half hour after analgesic administration, COX-1 activity was close to zero and remained significantly lower than before drug administration for 24 hours in all groups. The inhibition of COX-2 activity was significant for 30 minutes in all groups and up to 4 hours in group M. No alterations in laboratory tests or significant adverse effects were observed. Pain scores and need for rescue analgesia did not differ statistically among groups.

Conclusions Dipyrone at both doses and meloxicam provided a nonselective inhibition of COX-1 and -2 activities and effective analgesia without causing significant adverse effects or laboratory tests alterations.

Clinical relevance Dipyrone at both doses provides equally effective analgesia without causing adverse effects in cats undergoing ovariohysterectomy.

Keywords analgesia, COX, feline, metamizole, meloxicam.

Introduction

Dipyrone, also known as metamizole, is a drug used in some European and Latin American countries for its analgesic, antipyretic and spasmolytic effects (Levy et al. 1995). Although introduced in pharmacotherapy in 1922, the mechanism of action is not yet completely understood (Kötter et al. 2015). Previous studies have reported its action on different receptors: stimulation of the arginine—NO—cGMP pathway (Duarte et al. 1992); central action through inhibition of cyclooxygenase-3 (COX-3) (Chandrasekharan et al. 2002); endogenous opioid system (Vazquez et al. 2005); inhibition of COX-1 and COX-2 (Hinz et al. 2007) and endogenous cannabinoid system (Escobar et al. 2012).

Dipyrone may be a safe choice compared with opioids and nonsteroidal anti-inflammatory drugs (NSAIDs). However, dipyrone has been withdrawn from the market in several countries

because it has been associated with agranulocytosis in humans (Kötter et al. 2015). Studies investigating this association are heterogeneous and some results carry significant biases, such as small sample size, presence of comorbidities and the combination of dipyrone with other drugs (Andrade et al. 2016). The estimated mortality from agranulocytosis, aplastic anemia, anaphylaxis and gastrointestinal complications after short-term dipyrone use is reported as similar to that of paracetamol and lower than that of diclofenac and aspirin (Andrade et al. 1998).

In veterinary medicine, literature on this drug is sparse. Pharmacokinetics studies of dipyrone (25 mg kg^{-1}) administered by various routes in cats (Lebkowska-Wieruszewska et al. 2018) and dogs (Giorgi et al. 2018) have been published. Clinical studies have demonstrated analgesia efficacy from dipyrone in several scenarios in dogs (Martins et al. 2010; Imagawa et al. 2011; Flôr et al. 2013; Teixeira et al. 2013; Zanuzzo et al. 2015a). Only one published study was identified that used dipyrone for pain management in cats. In that study, dipyrone (25 mg kg^{-1} every 24, 12 or 8 hours for 5 days) combined with tramadol (2 mg kg^{-1} every 8 hours) administered intravenously (IV) after ovariohysterectomy provided effective postoperative pain management with no significant adverse effects, biochemical alterations or erythrocyte oxidation (Teixeira et al. 2019).

In domestic animals, there are no reports of significant adverse effects or laboratory tests alterations associated with dipyrone, and a study reported no renal, hepatic, bleeding time or bone marrow function alterations in dogs (Sarchahi et al. 2017). Regarding gastrointestinal effects, no pathological changes in the gastrointestinal tract of control rats or rats with preexisting induced gastric ulcers were observed after dipyrone administration (Berenguer et al. 2002). In addition, there are reports of increases in gastric mucus and gastric prostaglandin E_2 (PGE_2) (Batu & Erol 2007) and less gastric PGE_2 reduction than from piroxicam or celecoxib (Berenguer et al. 2002). However, the mechanisms of a protective effect by dipyrone on the gastric mucosa are still unclear (Collares & Troncon 2019).

The aim of this study was to evaluate the effect of dipyrone on COX-1 and COX-2 activities, to assess postoperative pain in cats undergoing elective ovariohysterectomy after IV administration of dipyrone or meloxicam during the first 24 hours and to identify any adverse effects occurring in 7 or 4 days after surgery with dipyrone or meloxicam, respectively. Our hypothesis was that dipyrone may inhibit COX with few adverse effects and provide postoperative analgesia similar to meloxicam.

Materials and methods

Experimental design

This prospective, randomized, blinded, controlled study was conducted at the Veterinary Teaching Hospital of the School of Veterinary Medicine and Animal Science (FMVZ), University of São Paulo (USP), Brazil. The study protocol was approved by the Institutional Animal Ethics Committee (8461060715) and a written informed consent was obtained from the owners before inclusion of the animals.

Animals

A total of 30 female domestic cats undergoing ovariohysterectomy were studied. All animals were deemed healthy and with an American Society of Anesthesiologists physical status I based on medical history, physical examination, complete blood count (CBC) and serum chemistry tests. Acclimatization occurred in two visits at the hospital before the beginning of the study, when animals were also introduced to the environment, cage, observers and sedation and pain assessment tools. Exclusion criteria included excessive fear or aggressiveness, signs of pre-existing pain, pregnancy, underlying diseases and recent use of anti-inflammatory drugs.

Anesthesia

Cats were administered acepromazine (0.1 mg kg^{-1}; Acepran 0.2%; Vetnil Indústria Comércio Produtos Veterinários Ltda, SP, Brazil) intramuscularly as premedication. After 15 minutes, a 22 gauge catheter (Angiocath; Becton Dickinson, SP, Brazil) was placed in a cephalic vein and anesthesia was induced with IV propofol (5–8 mg kg^{-1}; Propovan; Cristália Produtos Químicos e Farmacêuticos Ltda, SP, Brazil); maintenance was accomplished with isoflurane (Isoflorane; Cristália Produtos Químicos e Farmacêuticos Ltda) in 60–70% oxygen (air-oxygen mixture) administered through a rebreathing circuit. Pressure-controlled ventilation (Anesthesia Machine Inter Linea A; Intermed, SP, Brazil) was instituted with a peak inspiratory pressure of 7–8 cmH_2O, tidal volume 8 mL kg^{-1}, inspiratory:expiratory ratio of 1:2 and positive end-tidal expiratory pressure 1 cmH_2O. The respiratory rate (f_R) was adjusted to maintain end-tidal carbon dioxide partial pressure within 30–40 mmHg (5.0–6.0 kPa). End-tidal isoflurane concentration and inspired oxygen concentration were measured using a side stream gas analyzer (POET IQ2; Criticare Technologies Inc., WI, USA). Heart rate (HR), rhythm (DX 2020 multiparametric monitor; Dixtal Biomédica Indústria e Comércio Ltda, SP, Brazil) and Doppler systolic arterial pressure (SAP; Doppler 811-B; Parks Medical Electronics, OR, USA) were monitored during the entire procedure. Intraoperative IV fluid therapy was lactated Ringer's solution (3 mL kg^{-1} $hour^{-1}$).

A remifentanil constant rate infusion (CRI; 0.2 μg kg^{-1} $minute^{-1}$; Ultiva 2 mg; GlaxoSmithKline Brasil Ltda, RJ, Brazil) was administered IV with a syringe driver (ST670 syringe pump; Samtronic, SP, Brazil) starting after induction of

anesthesia and finishing during abdominal closure. If SAP and/or HR increased or decreased by >15% of values immediately before skin incision, remifentanil CRI was increased or decreased, respectively, by 0.1 μg kg^{-1} $minute^{-1}$.

Cats were randomly assigned by an online random sequence generator (http://www.randomization.com) to one of three groups, 10 cats in each. At 10 minutes before the end of the remifentanil CRI, during skin closure, cats were administered IV analgesics according to the assigned group: group D25, dipyrone (25 mg kg^{-1} every 24 hours; D500; Zoetis Inc., SP, Brazil); group D12.5, dipyrone (12.5 mg kg^{-1} every 12 hours); or group M, meloxicam (0.1 mg kg^{-1} every 24 hours; Maxicam 0.2%; Ouro Fino Saúde Animal, SP, Brazil). The drugs were diluted in 0.9% saline solution to a final volume of 0.5 mL for each group. A collaborator prepared the injections so that the evaluator would be unaware of the group assignment. In the first 24 hours after anesthesia, the drugs were administered IV. At hospital discharge, the clients were directed to administer the same drug orally for 6 days (dipyrone) or 3 days (meloxicam).

All the procedures were performed by a single anesthesiologist (LAG) and a single senior surgeon (JMM) with an assistant using a standardized surgical protocol.

Measurement of *ex vivo* COX activity

Ex vivo COX-1 and COX-2 activities were determined by measuring concentrations of their major enzymatic products [thromboxane B_2 (TXB_2) and PGE_2, respectively], according to an adaptation of a previously described technique (Duz et al. 2015). Blood samples (2 mL) were drawn from the cephalic vein catheter of six randomly selected cats per group and were transferred to a heparin tube (MiniCollect heparin 1 mL; Greiner Bio-One Ltda, SP, Brazil) and a glass tube at the following time points: immediately before the first analgesic administration and at 30 minutes, 4 and 24 hours. At 24 hours, blood was collected before the next drug administration.

For TXB_2 analysis, immediately after collection, 1 mL of whole blood in the glass tube was incubated at 37 °C for 1 hour. Afterwards, the blood was centrifuged at 3000 ***g*** for 5 minutes at 4 °C and 100 μL of the supernatant plasma was harvested, added to 400 μL of methanol and homogenized. This solution was then centrifuged at 6000 ***g*** for 10 minutes and stored at −80 °C until COX-1 activity was determined by means of a technique previously described (Brideau et al. 2001).

For *ex vivo* COX-2 activity, heparinized blood (1 mL) was immediately transferred into two 1.5 mL polypropylene tubes (500 μL each). One tube contained 10 μL of lipopolysaccharide isolated from *Escherichia coli* 0111:B4 in 0.1% bovine serum albumin in phosphate buffered saline (PBS) solution. The second tube contained 10 μL of PBS and was used as the negative control sample. Samples were then incubated at 37 °C for 24 hours. Afterwards, samples were centrifuged at 2000 ***g*** for 5 minutes at 18 °C, 100 μL of plasma were added to 400 μL of methanol and homogenized. This solution was then centrifuged at 6000 ***g*** for 10 minutes at 18 °C; supernatant was harvested and stored at −80 °C until the time of PGE_2 concentration analysis.

All tests were performed in duplicate and the quantification of TXB_2 and PGE_2 concentrations were performed with commercial enzyme-linked immunosorbent assay (ELISA) kits (Thromboxane B_2 ELISA kit and PGE_2 ELISA kit monoclonal; Cayman Chemical, MI, USA) according to the manufacturer's instructions.

Laboratory tests

Blood samples (2 mL) were collected for measurement of hepatic function tests (alanine transaminase; alkaline phosphatase; aspartate aminotransferase; gamma-glutamyltransferase; serum albumin and total protein) and renal function tests (creatinine; urea) 7 days before and 7 days after anesthesia (clinical laboratory of the Veterinary Teaching Hospital of FMVZ-USP). Symmetric dimethylarginine (SDMA) concentration was also measured at 7 days before, 24 hours and 7 days after anesthesia in samples from all cats used in the COX inhibition evaluation (IDEXX Laboratories Inc., ME, USA).

Adverse effects

Adverse effects and short-term postoperative complications were monitored in the 24 hours after first analgesic administration and reassessed on postoperative days 7 and 10 (suture removal). Through daily telephone contact, the owners were asked about their cats' general condition, presence of emesis, sialorrhea, diarrhea, changes in food and water intake, abnormal behavior and general aspect of the surgical wound.

Physiological variables

Measurements of HR, f_R, rectal temperature (RT) and blood glucose concentration (Glucometer Optium Xceed; Abbott Laboratories, Philippines) were obtained immediately before the premedication (baseline) and 1, 2, 4, 8, 12 and 24 hours after first analgesic administration.

Pain and sedation assessment

Pain and sedation were evaluated at the same time points as the physiological variables. Subjective pain scores were obtained from four pain scales; the visual analogue scale (VAS) (Jensen et al. 2003), Colorado State University feline acute pain scale in English (CSU-FAPS) (Hellyer et al. 2006), UNESP-Botucatu multidimensional composite pain scale for assessing postoperative pain in cats in Portuguese (MCPS) (Brondani

et al. 2012) and Glasgow feline composite measure pain scale (CMPS-Feline) (Reid et al. 2017). Sedation was evaluated using a scale (0−3; no effect, mild, moderate and severe) adapted from Valverde et al. (2004).

Rescue analgesia

Rescue medication tramadol hydrochloride (2 mg kg^{-1}; Cronidor 2%; Agener União Saúde Animal, SP, Brazil) was administered IV if CMPS-Feline scores were ≥5. Additional tramadol, 1 mg kg^{-1} IV, could be administered if analgesia was inadequate after 30 minutes. The person that performed pain assessment (MAAP) was unaware of the drug administered. Cats administered rescue medication were reassessed 30 minutes after tramadol administration and up to 24 hours; however, the physiological variables data, pain and sedation scores recorded after rescue analgesia were excluded from the analysis.

Statistical analysis

A sample size calculation was performed for *ex vivo* COX activity and pain assessment [COX-1 activity inhibition in humans after oral administration of 500 mg of dipyrone of 72.9 ± 5% (Hinz et al. 2007); need for rescue analgesia of 70% and 20% of dogs submitted to ovariohysterectomy and IV administration of 15 or 25 mg kg^{-1} of dipyrone (Imagawa et al. 2011), respectively].

Data are expressed as mean ± standard deviation (parametric variables) or median (range) (nonparametric variables) as appropriate. The data were tested for normality using a D'Agostino−Pearson test. A two-way analysis of variance was used to evaluate parametric data and a Kruskal−Wallis test was applied on nonparametric data. *Post hoc* analysis was performed with Tukey or Dunn tests to identify differences among time points and groups. The Spearman's rank correlation between pain scales was evaluated. The number of cats that required rescue analgesia was compared among groups using the Fisher exact probability test. Statistical significance was attributed if $p < 0.05$. Analyses were performed using GraphPad Prism Version 7.03 (GraphPad Software Inc., CA, USA).

Results

A total of 37 cats were initially selected for participating in the study. Overall, four cats were excluded because of noncompliant preoperative behavior and three cats were excluded because of pregnancy. Groups D25, D12.5 and M did not differ significantly regarding body weight (2.7 ± 0.5; 2.7 ± 0.4; 2.7 ± 0.5 kg, respectively), age (13 ± 5; 12 ± 9; 15 ± 13 months, respectively) and surgical time (28 ± 6; 25 ± 4; 30 ± 4 minutes, respectively).

Before analgesic drug administration, TXB_2 concentrations were not different among groups. After drug administration, concentrations were decreased at all time points in all groups ($p < 0.0001$; Fig. 1). However, there was a highly significant increase between 0.5 and 4 hours in M ($p = 0.0004$). TXB_2 concentrations were higher in M than D12.5 and D25 at 4 hours ($p = 0.003$ and $p < 0.0001$, respectively) and at 24 hours ($p = 0.007$ and $p = 0.011$, respectively).

PGE_2 concentrations did not differ among groups before drug administration. At 4 hours PGE_2 concentration was higher in D25 than in M ($p = 0.048$; Fig. 1). PGE_2 concentration had decreased in D12.5 at 0.5 hour ($p = 0.0001$) and 4 hours ($p = 0.011$); in D25 at 0.5 hour ($p < 0.0001$), 4 hours ($p = 0.0001$) and 24 hours ($p = 0.0004$); and in M at 0.5 and 4 hours ($p = 0.002$ and $p = 0.007$, respectively).

There were no significant differences regarding laboratory test results, except for SDMA. When compared with 7 days before anesthesia, SDMA concentration was lower at 24 hours in M ($p = 0.0122$) and higher at 7 days postoperatively than at 24 hours in D25 and M ($p = 0.038$ and $p = 0.026$, respectively; Fig. 2). There were no significant differences among groups. A high SDMA concentration (15 µg dL^{-1}) was measured in a single cat in M at 7 days.

Sialorrhea was observed in all cats administered dipyrone drops orally at home. No emesis or alterations in food and water intake were observed.

The f_R was not significantly different postoperatively among groups or time points (Table 1). HR did not differ among groups, but was increased at 1 hour compared with baseline in M ($p = 0.033$; Table 1). RT was decreased 1 hour after anesthesia compared with baseline in all groups. Blood glucose concentrations were not different among groups, but were higher than baseline at 4 hours in D25 ($p = 0.018$) and at 1 hour in M ($p = 0.020$; Table 1).

Pain scores did not differ significantly among groups; however, VAS scores were significantly higher than baseline in D12.5 at 4 hours ($p = 0.0415$; Fig. 3). A correlation between pain scales indicated a moderate (VAS × MCPS; $r = 0.4536$), strong (CSU-FAPS × MCPS; $r = 0.6634$; VAS × CMPS-F; $r = 0.7021$; MCPS × CMPS-F; $r = 0.7152$) or very strong association (VAS × CSU-FAPS; $r = 0.8313$). Sedation numbers were increased at 1 hour after first analgesic administration in all groups ($p < 0.0001$) (Table 2). There were no significant differences among groups regarding the need for rescue analgesia, even though the time of administration differed among groups (Table 3). A total of eight cats required a single tramadol administration; four, two and two in groups D25, D12.5 and M, respectively.

Discussion

Surprisingly, dipyrone at both doses inhibited COX-1 and COX-2 for 24 hours and 0.5 hour, respectively, in cats. However, no

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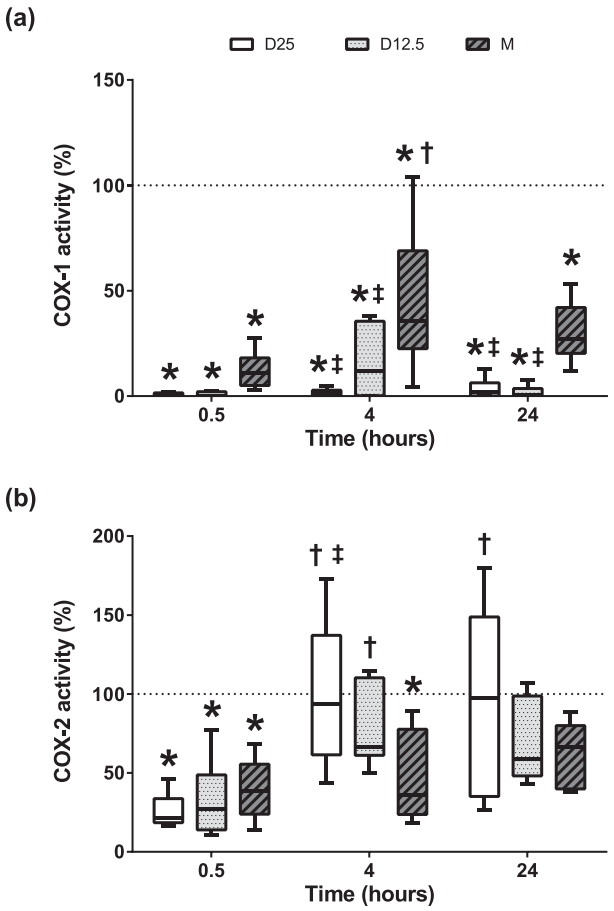

Figure 1 Inhibition of cyclooxygenase activity. (a) COX-1 and (b) COX-2 activity percentages are represented by Box plot [median (line), interquartile range (box) and range (whiskers)] in cats administered dipyrone (25 mg kg^{-1} every 24 hours; group D25) or dipyrone (12.5 mg kg^{-1} every 12 hours; group D12.5) or meloxicam (0.1 mg kg^{-1} every 24 hours; group M) at time points: time 0 immediately before the first analgesic administration, 0.5, 4 and 24 hours. The horizontal dotted line illustrates the 100% activity of cyclooxygenases, value obtained immediately prior to administration of the postoperative medication. COX, cyclooxygenase. *Significantly different from baseline within the same group ($p < 0.01$). †Significantly different from 0.5 hour within the same group ($p < 0.01$). ‡Significantly different from group M at the same time point ($p < 0.05$).

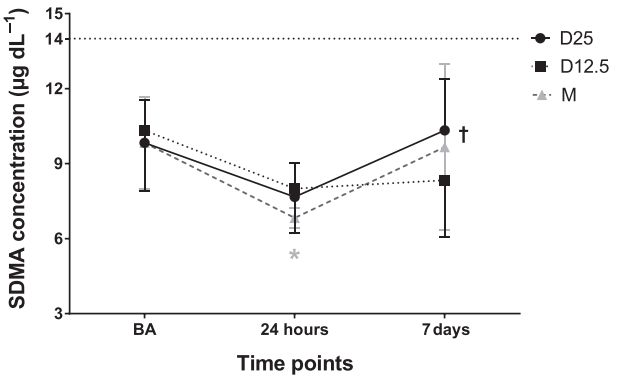

Figure 2 Symmetric dimethylarginine (SDMA) plasma concentrations in cats administered dipyrone (25 mg kg^{-1} every 24 hours; group D25) or dipyrone (12.5 mg kg^{-1} every 12 hours; group D12.5) or meloxicam (0.1 mg kg^{-1} every 24 hours; group M). Time points: 7 days before anesthesia (BA), 24 hours and 7 days after anesthesia. The horizontal dotted line represents the upper limit of normal SDMA reference interval in cats. Data are from six cats in each group presented as mean ± standard deviation. *Significantly different from before anesthesia within the same group, M ($p < 0.05$). †Significantly different from 24 hours within the same group, D25 ($p < 0.05$).

significant adverse effects and alterations in laboratory tests were observed throughout the study. In addition, dipyrone was as effective as meloxicam for postoperative analgesia.

During the literature search, no previous papers evaluating the effect of dipyrone on COX activity in cats were found. This study was based on the one performed by Duz et al. (2015), which described this technique in horses.

The administration of the drugs led to an inhibition of COX-1 activity, as observed by the plasma TXB_2 concentration decrease. These results corroborate previous studies on the use of dipyrone (Hinz et al. 2007; Pierre et al. 2007) in other species and meloxicam in other species (Engelhardt et al. 1996a, b; Brideau et al. 2001) and in cats (Giraudel et al. 2005).

The COX-1 activity inhibition was greater at both dipyrone doses than meloxicam from 4 hours on. Even though this inhibition is similar to that caused by nonselective NSAIDs, dipyrone was not associated with any adverse effects commonly observed with NSAIDs. A multicentric study of humans reported higher gastrointestinal safety with dipyrone than classical NSAIDs (Laporte et al. 1991) and its low acidity may be responsible for the favorable gastrointestinal tolerance (Hinz et al. 2007).

Despite the significant COX-1 inhibition seen in this study, no postoperative bleeding was observed in any cat. In a similar study in dogs, a single dose of dipyrone (25 mg kg^{-1} IV) alone or in combination with meloxicam (0.2 mg kg^{-1} IV) inhibited platelet aggregation for 3 and 5 hours, respectively, with no alterations in thromboelastometry and buccal mucosal bleeding time (Zanuzzo et al. 2015b).

The *ex vivo* evaluation of COX-2 activity inhibition showed that it occurred in all groups, similar to results from studies in other species (Engelhardt et al. 1996a, b; Brideau et al. 2001; Giraudel et al. 2005; Hinz et al. 2007; Pierre et al. 2007). At 0.5 hour, PGE_2 concentrations had decreased significantly in all groups, lasting longer (4 hours) in group M.

In the present study, both dipyrone doses strongly inhibited both COX isoforms in a nonselective way; COX-1 inhibition was greater and longer lasting than that of COX-2. The COX

Table 1 Mean ± standard deviation of heart rate (HR), respiratory rate (f_R), rectal temperature (RT) and blood glucose concentrations in cats after ovariohysterectomy. Cats were anesthetized with isoflurane and remifentanil constant rate infusion for the surgical procedure, and for recovery were administered dipyrone (25 mg kg^{-1} every 24 hours; group D25); dipyrone (12.5 mg kg^{-1} every 12 hours; group D12.5); or meloxicam (0.1 mg kg^{-1} every 24 hours; group M), intravenously. Data are from 10 cats in each group. Baseline, immediately before the premedication

Variable	Group	Time (hours)						
		Baseline	1	2	4	8	12	24
HR (beats $minute^{-1}$)	D25	220 ± 44	235 ± 20	240 ± 26	214 ± 16	200 ± 17	204 ± 21	191 ± 19
	D12.5	214 ± 30	239 ± 41	219 ± 37	220 ± 30	215 ± 25	214 ± 23	207 ± 26
	M	208 ± 28	241 ± 22*	215 ± 26	207 ± 17	206 ± 26	202 ± 20	205 ± 29
f_R (breaths $minute^{-1}$)	D25	59 ± 12	47 ± 12	46 ± 8	45 ± 11	42 ± 10	40 ± 6	46 ± 10
	D12.5	53 ± 13	46 ± 16	43 ±13	43 ± 10	42 ± 10	41 ± 6	45 ± 10
	M	56 ± 19	52 ± 26	48 ± 13	52 ± 20	54 ± 12	52 ± 14	56 ± 12
RT (°C)	D25	38.3 ± 1	36.3 ± 1†	37.6 ± 0	38.1 ± 0	38.1 ± 0	38.1 ± 1	37.9 ± 0
	D12.5	38.4 ± 0	36.7 ± 1†	37.6 ± 1	38 ± 0	38.3 ± 0	38.2 ± 0	38.2 ± 1
	M	38.5 ± 0	36.6 ± 1†	37.8 ± 1	38.6 ± 1	38.3 ± 1	37.9 ± 0	37.8 ± 0
Blood glucose (mg dL^{-1})	D25	81 ± 13	96 ± 20	85 ± 15	105 ± 19*	81 ± 12	84 ± 13	75 ± 13
	D12.5	104 ± 31	108 ±24	105 ± 20	123 ± 24	91 ± 21	95 ± 21	90 ± 18
	M	78 ± 16	103 ± 34*	95 ± 22	92 ± 14	84 ± 7	81 ± 9	79 ± 12

*Significantly different from baseline within the same treatment ($p < 0.05$).
†Significantly different from baseline within the same treatment ($p < 0.01$).

selectivity ratio was not calculated because *ex vivo* assays cannot be used for these estimates (Duz et al. 2015). Hinz et al. (2007) observed that 4-methylamino antipyrine, an active metabolite of dipyrone, led to a substantial COX inhibition of both isoforms equally.

Meloxicam also inhibited both COX isoenzymes, however inhibition of COX-1 by meloxicam was less intense than from dipyrone, whereas COX-2 inhibition was longer lasting. Previous studies, including assays of canine and feline whole blood after administration of meloxicam, identified only a slight COX-2 selectivity (COX-1:COX-2 ratio of 10 and 3.5, respectively) (Brideau et al. 2001; Giraudel et al. 2005). Blood collected from 10 cats revealed that meloxicam inhibited COX-1 by at least 20% at minimal plasma concentrations and exceeded 40% at therapeutic plasma concentrations (Giraudel et al. 2005). These results are similar to those in the present study.

Despite the nonselective COX-2 inhibition, the drugs and dosages administered in the present study resulted in no adverse effects or alterations in laboratory tests (CBC and serum chemistry) throughout the evaluation period. In group M, serum SDMA concentrations decreased at 24 hours compared with 7 days before and 7 days after anesthesia. In group D25, they were significantly lower at 24 hours compared with 7 days postoperatively. However, the changes showed a similar pattern in all groups, and the values did not exceed the feline normal reference interval (0–14 μg dL^{-1}). These results suggest that glomerular filtration rate increased after the surgical procedure, probably influenced by intraoperative fluid therapy. There were no differences among groups in concentrations of SDMA, however, one cat in M presented a high value (15 μg dL^{-1}) at 7 days postoperatively.

Pain is a vital sign that has a complex pathophysiology. Sensitization of nociceptors or hyperalgesia is a common finding in inflammatory pain and is a consequence of the action of inflammatory mediators such as PGE_2. These mediators lower the threshold of excitability of receptors, facilitating the activity of notoriously painful substances, such as histamine and bradykinin (Zhang et al. 1997). NSAIDs, such as meloxicam, have a proven analgesic potential related to COX inhibition, decreasing the production of PGE_2 in cats (Giraudel et al. 2005).

Although dipyrone is not a new drug, the mechanism of action is complex and not yet completely understood. Based on studies in other species, it was hypothesized that effects of dipyrone depend at least partially on COX inhibition and consequently on the reduction in PGE_2 synthesis peripherally (Abbate et al. 1990; Hinz et al. 2007). Our findings corroborate these data; however, it is likely that dipyrone also produces analgesia through other mechanisms in cats. Chandrasekharan et al. (2002) reported the existence of a splicing variant of COX-1, referred to as COX-3, and suggested that dipyrone selectively inhibits this enzyme. However, that *in vitro* study used constructs of canine COX-3 and murine COX-1 and -2 with disparate activity levels, leading to uncertainties and controversies (Hinz et al. 2007). In addition, there is no evidence regarding the presence, physiological role and dipyrone selectivity for COX-3 in the feline species.

Pain scores did not differ significantly among groups, indicating that all analgesic protocols were effective in controlling

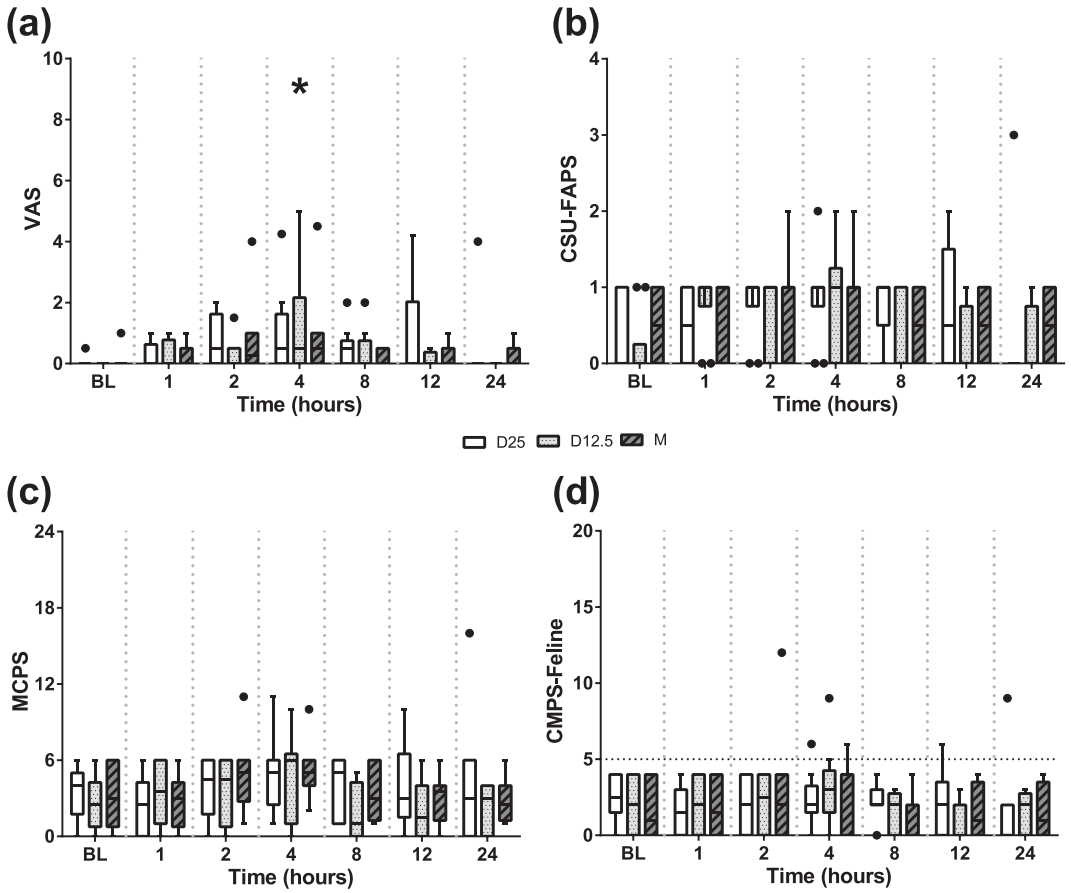

Figure 3 Pain scores for (a) visual analogue scale (VAS), (b) Colorado State University feline acute pain scale (CSU-FAPS), (c) UNESP-Botucatu multidimensional composite pain scale for assessing postoperative pain in cats (MCPS), and (d) Glasgow feline composite measure pain scale (CMPS-Feline) in cats administered dipyrone (25 mg kg^{-1} every 24 hours; group D25, white column) or dipyrone (12.5 mg kg^{-1} every 12 hours; group D12.5, stippled column) or meloxicam (0.1 mg kg^{-1} every 24 hours; group M, hatched lines). Time points: baseline (BL), 1, 2, 4, 8, 12 and 24 hours after first analgesic administration. Data are from 10 cats in each group and presented as median (line), interquartile range (box) and range (whiskers). The horizontal dotted line in (d) represents the rescue analgesia cut-off score. *Significantly different from baseline within the same treatment ($p < 0.05$).

ovariohysterectomy postoperative pain. Although monotherapy with dipyrone or an NSAID is indicated for mild pain only (WHO 1987), an experienced surgeon performing ovariohysterectomy in young animals with an ideal body condition score may produce less surgical trauma and thus mild pain, corroborating the findings of previous studies (Imagawa et al. 2011; Zanuzzo et al. 2015a; Pereira et al. 2018).

To find the lowest effective analgesic dose yielding the least adverse effects, a low dose (12.5 mg kg^{-1}) was tested and compared with the commonly used dose for dogs and cats. There were no significant differences among groups regarding the need for rescue analgesia (40%, 20% and 20% in D25, D12.5 and M, respectively), similar to a study in dogs undergoing ovariohysterectomy administered either dipyrone, meloxicam or a combination of these drugs (Zanuzzo et al. 2015a). The administration of tramadol as rescue analgesia was adequate to reduce scores in all pain scales in the present study.

The four subjective pain scales were used to measure postoperative pain more precisely and to analyze correlations among them. All cats requiring rescue analgesia were assigned scores above the cut-off value for rescue in all scales and a moderate (VAS × MCPS) to very strong (VAS × CSU-FAPS) correlation among them was found. A strong association between validated scales, MCPS and CMPS-FS scores ($p < 0.0001$), was observed, corroborating the findings of Steagall et al. (2018). However, only VAS displayed a significant difference between baseline and 4 hours, when two cats in group D12.5 needed rescue analgesia.

This study had limitations. First, because the surgical procedure was performed by an experienced surgeon, results may not be extrapolated for the same surgery performed under

Table 2 Median (range) sedation scores according to a sedation scale (0−3; no effect, mild, moderate and severe) in cats administered intravenous dipyrone 25 mg kg^{-1} every 24 hours (group D25) or 12.5 mg kg^{-1} every 12 hours (group D12.5) or meloxicam 0.1 mg kg^{-1} every 24 hours (group M) after anesthesia for ovariohysterectomy

Group	Time (hours)						
	Baseline	1	2	4	8	12	24
D25	0 (0−0)	1 (1−2)*	0.5 (0−1)	0 (0−0)	0 (0−0)	0 (0−0)	0 (0−0)
D12.5	0 (0−0)	1 (1−2)*	0 (0−1)	0 (0−0)	0 (0−0)	0 (0−0)	0 (0−0)
M	0 (0−0)	1 (1−1)*	0 (0−1)	0 (0−0)	0 (0−0)	0 (0−0)	0 (0−0)

*Significantly different from baseline within the same treatment ($p < 0.01$).

Table 3 Rescue analgesia administered to cats in the first 24 hours after ovariohysterectomy. Cats were anesthetized with isoflurane and remifentanil constant rate infusion for the surgical procedure, and for recovery were administered intravenously (IV) dipyrone (25 mg kg^{-1} every 24 hours; group D25); dipyrone (12.5 mg kg^{-1} every 12 hours; group D12.5); or meloxicam (0.1 mg kg^{-1} every 24 hours; group M). Data are from 10 cats in each group. Baseline, immediately before the premedication. Rescue medication (tramadol hydrochloride, 2 mg kg^{-1} IV) was administered if Glasgow feline composite pain scale scores were ≥5

Group	Time (hours)								Median administrations per animal
	Baseline	1	2	4	8	12	24	Total	
D25	0	0	0	1	0	2	1	4 (40%)	1
D12.5	0	0	0	2	0	0	0	2 (20%)	1
M	0	0	1	1	0	0	0	2 (20%)	1

different conditions including less experience and longer duration of anesthesia. Second, the 20 hour interval between blood collection for *ex vivo* COX activity analysis may have missed some information, such as the exact duration of the COX-2 inhibition by meloxicam. Third, meloxicam was prescribed for only 4 days postoperatively, whereas dipyrone was prescribed for 7 days. The duration of drug administration followed our standard meloxicam protocol for management of postoperative ovariohysterectomy pain in cats and the main objective of this study was to evaluate the short-term effects of dipyrone.

Conclusion

In the present study, dipyrone (12.5 and 25 mg kg^{-1} for 7 days) and meloxicam (0.1 mg kg^{-1} for 4 days) were administered after ovariohysterectomy in cats. The results indicated adequate analgesia provided by all protocols and that both dipyrone regimens were equally effective. Unexpectedly, both dipyrone doses strongly inhibited COX-1 and COX-2. Laboratories tests of CBC and hepatic and renal function were unchanged and no significant adverse effects were observed. Further trials are indicated to study the mechanism of action of dipyrone and the anti-inflammatory effect of this drug.

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Authors' contributions

MAAP: study design, data management and interpretation, statistical analysis, preparation of the manuscript. KDC: data management and English review. LAG, RSTS, PBF and AMA: data management. DAO: data interpretation, statistical analysis. COMSG: study design, data interpretation, manuscript revision. DTF: study design, funding obtention, data management and interpretation, preparation of the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

Authors declare no conflict of interest.

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