

The Interaction between Orally Administered Non-Steroidal Anti-Inflammatory Drugs and Prednisolone in Healthy Dogs

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ABSTRACT. The interaction between oral non-steroidal anti-inflammatory drugs (NSAIDs) and prednisolone administered concurrently for 30 days was studied in 18 healthy dogs divided into 3 groups of 6 dogs each: a drug-free negative control group (NC group) given 2 gelatin capsules; a group given meloxicam (0.1 mg/kg) and prednisolone (0.5 mg/kg) (MP group); and a group given a reduced dosage of ketoprofen (0.25 mg/kg, PO) and prednisolone (0.5 mg/kg, PO) (KP group). The dogs were periodically monitored by physical examinations, blood analyses, endoscopic examinations, fecal occult blood tests, renal function tests [effective renal plasma flow (ERPF) and glomerular filtration rate (GFR)], urinalyses [urinary sediments, and urinary micro-albumin to creatinine ratio (UAlb/Cre)], urinary enzyme indices, and haemostatic function tests [buccal mucosa bleeding time (BMBT), cuticle bleeding time (CBT)]. Significant changes were observed in the KP group, including a decrease of ERPF and GFR, an increased UAlb/Cre ratio, prolonged BMBT and CBT, as well as the presence of more severe grades of endoscopic lesions and fecal occult blood. In both the MP and KP groups, abnormal enzymuria with exfoliation of renal tubular epithelial cells in the urine was found. However, no significant changes in any of the other tests were observed in the MP group compared with the NC group. These findings suggest that the combination of NSAIDs, even selective COX-2 inhibitors, with prednisolone may be contraindicated due to the potential for serious adverse effects on the kidneys, the platelets, and the gastrointestinal tract.

KEY WORDS: canine, interaction, ketoprofen, meloxicam, prednisolone.

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Non-steroidal anti-inflammatory drugs (NSAIDs) have been commonly used for the management of chronic pain due to inflammatory joint disease in dogs [15, 18, 32]. The analgesic and anti-inflammatory effects of NSAIDs have been attributed to the prevention of prostaglandin (PG) synthesis from arachidonic acid, through inhibition of cyclooxygenase (COX) [18]. Two isoenzymes of COX (COX-1 and COX-2) have been identified. COX-1 is expressed ubiquitously in many tissues and is considered to be associated with the biosynthesis of prostaglandins that have a role in physiological homeostasis within the body, in particular in the stomach and the kidneys, as well as with respect to platelet function. On the other hand, COX-2 is a cytokine-inducible isoenzyme and its expression is induced in inflammatory tissues. COX-2 also produces PGs that induce hyperalgesia by sensitizing sensory nerve endings to various other mediators, such as bradykinin, histamine, and substance P [37].

Racemic ketoprofen, an NSAID of the propionic acid group, has a potent analgesic effect in dogs [15]. Ketoprofen is a relatively selective COX-1 inhibitor [20, 33, 41]; adverse effects such as mild to moderate gastric mucosal injuries and renal hypofunction have been described [30, 31]. On the other hand, meloxicam, an NSAID of the oxamicam group, has a comparable analgesic effect to ketoprofen in dogs [5], and is also approved in Japan for the treatment

of chronic inflammatory musculoskeletal conditions in dogs [32]. Meloxicam is a selective COX-2 inhibitor [20, 33] and exhibits markedly reduced gastrointestinal tract adverse effects [10]. It is currently thought that the undesirable adverse effects of NSAIDs are due to inhibition of COX-1, especially in the gastrointestinal tract, and that inhibition of COX-2 is mainly responsible for pain control, due to its pathophysiological role in pain [37].

Corticosteroids, such as prednisolone, are also commonly used in several inflammatory diseases, including osteoarthritis [18], and are well known to cause gastric mucosal injury in dogs [34, 35]. Clinically, NSAIDs and corticosteroids are frequently used alone. However, they are sometimes co-administered, despite this being contraindicated. In fact, severe adverse effects, such as gastric ulcer and perforation, have been reported in dogs given combination treatment with NSAIDs and corticosteroids [24].

Well-known adverse effects of NSAIDs include renal insufficiency and inhibition of platelet aggregation [23]. However, little is known about the adverse effects on the kidneys and platelet function, as well as the gastrointestinal tract, caused by combining NSAIDs with corticosteroids in healthy dogs. The present study was therefore designed to investigate the effects of combination treatment with NSAIDs and corticosteroids on not only the gastrointestinal tract but also on the kidneys and platelet function in healthy Beagle dogs. We choose to use ketoprofen as a relatively selective COX-1 inhibitor, meloxicam as a selective COX-2 inhibitor, and prednisolone as the corticosteroid.

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MATERIALS AND METHODS

Dogs: Eighteen healthy Beagle dogs (intact females) were used in the present study. The dogs ranged from 13 to 60 months of age (mean \pm SD; 24.6 ± 10.5 months) and weighed between 7.2 and 10.5 kg (mean \pm SD; 8.3 ± 0.9 kg). The study was approved by the Animal Research Committee of Iwate University.

Experimental design: The dogs were randomly assigned to three treatment groups. The dogs in the negative control (NC) group (6 females, body weight 8.45 ± 1.33 kg, Nos. 1–6) received 2 empty gelatin capsules PO, q 24 hr (empty gelatin capsules, Kobayashi Capsulae Inc., Hyogo, Japan) as a drug-free negative control. The dogs in the meloxicam and prednisolone (MP) group (6 females, body weight 8.13 ± 0.63 kg, Nos. 7–12) received meloxicam (Metacam, Nippon Boehringer Ingleheim, Co Ltd., Hyogo, Japan) (0.1 mg/kg, PO, q 24 hr) and prednisolone (Prednisolone, Takeda Chemical Industries, Ltd., Osaka, Japan) (0.5 mg/kg, PO, q 24 hr). The dogs in the reduced dosage ketoprofen and prednisolone (KP) group (6 females, body weight 8.25 ± 1.07 kg, Nos. 13–18) received racemic ketoprofen (Ketofen, Merial Japan Co., Ltd., Fukushima, Japan) (0.25 mg/kg, PO, q 24 hr) and prednisolone (0.5 mg/kg, PO, q24 hr). Treatment was given at 8:00–9:00 a.m. daily for 30 days. All experimental dogs were housed in cages and fed commercial dog food once a day after treatment in the morning. Water was given *ad libitum*. Clinical signs including anorexia, vomiting, diarrhea, depression or abdominal pain were recorded and physical examinations were performed on both groups once before and then every day after the start of treatment. Once before and every 10 days after the start of treatment, venous blood analyses, including CBC and serum biochemical analysis, including alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase (GGT), BUN, creatinine (Cre), lactate dehydrogenase, total cholesterol, total protein, albumin, globulin, total bilirubin, calcium, and phosphate, were done using an automatic analyzer (Hitachi Automatic Analyzer 7060, Hitachi Inc., Tokyo, Japan) in each group.

Endoscopic examination: Endoscopic examination of the gastrointestinal mucosa was done in each group before and every 7 days after the start of treatment. Food was withheld for 24 hr and water was withheld for 6 hr before anesthesia. Anesthesia was induced with propofol (6 mg/kg, IV bolus), and intubation with an intratracheal tube was done. Anesthesia was maintained with isoflurane in oxygen for the duration of the endoscopic examination. The gastrointestinal tract was examined using a videoendoscope (Olympus ves, Olympus Avs Co., Ltd., Tokyo, Japan). Photographs of the mucosa were taken at the following 8 sites: esophagus, cardia/fundus, stomach body, body-antrum junction, angular ventriculi, pyloric antrum, pylorus, and duodenum. The number and size of mucosal lesions were recorded and graded 0–6 according to the scale reported by Forsyth *et al.* [9, 10]: grade 0, no punctate erosions and/or hemorrhages lesions; grade 1, 1–5 punctate erosions and/or hemorrhages;

grade 2, 6–15 punctate erosions and/or hemorrhages lesions; grade 3, 16–25 punctate erosions and/or hemorrhages lesions; grade 4, more than 25 punctate erosions and/or hemorrhages and 1 to 5 invasive erosions; grade 5, more than 5 invasive erosions; and grade 6, ulcers. Based on these criteria, invasive erosions were defined as extensive hemorrhages or erosions with evidence of invasion as indicated by having a detectable depth/breadth significantly greater than a pinhead-sized discontinuation of the mucosal epithelium. An ulcer was defined as a lesion producing a wide discontinuation of the mucosa and having a crater-like center.

Fecal occult blood tests: A test for fecal occult blood in fresh feces was done in each group before and on day 30 after treatment started. A commercially available kit (Occult Blood Slide Shionogi II, Shionogi Co., Ltd., Osaka, Japan) that included the tetramethylbenzidine and guaiac methods was used. The analysis was done and the results were graded according the manufacturer's instructions. In the grading scale, grade 0 indicated negative fecal occult blood, grade 1 indicated that the sample was weakly positive, grade 2 indicated that the sample was positive, grade 3 indicated that the sample was moderately positive, and grade 4 indicated that the sample was strongly positive.

Renal function tests: Renal function was assessed in the awake condition by determining the effective renal plasma flow (EPRF) and the glomerular filtration rate (GFR) in each group before and every 10 days after the start of treatment. Para-aminohippurate sodium (PAH) (P-aminohippurate sodium, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) clearance (Cl_{PAH}) and endogenous creatinine clearance (Cl_{Cre}) were measured to assess EPRF and GFR, respectively. Cl_{PAH} and Cl_{Cre} were analyzed simultaneously using the methods reported by Narita *et al.* [30]. Briefly, Cl_{PAH} was analyzed by bolus administration of PAH (40 mg/kg, IV). Blood was collected at 30 min before PAH administration, and 35 and 65 min after PAH administration. Urine was collected aseptically by a sterilized silicone elastomer-coated Foley catheter from 35 to 75 min after PAH administration. Cl_{PAH} and Cl_{Cre} were estimated using the following formulae:

$$Cl_{PAH} \text{ (ml/min/kg)} = \frac{\text{Urine}_{PAH} \text{ (mg/dl)} \times \text{Volume (ml/min/kg)}}{\text{Plasma}_{PAH} \text{ (mg/dl)}}$$

$$Cl_{Cre} \text{ (ml/min/kg)} = \frac{\text{Urine}_{Cre} \text{ (mg/dl)} \times \text{Volume (ml/min/kg)}}{\text{Plasma}_{Cre} \text{ (mg/dl)}}$$

Urinalyses: Urinalysis, including specific gravity, dipstick analysis, and urinary sediment examination was performed in each group before and every 5 days after starting treatment. Urinalysis was performed using a urine dipstick (Aution Sticks 5EA, Arkray Inc., Kyoto, Japan) and the specific gravity of the urine was assayed using a refractometer (Clinical Refractometer T2-NE, Atago Co., Ltd., Tokyo, Japan). Microscopic evaluation of the urine was done on urine sediment stained with the Sternheimer-Malbin stain (URI-CEL, Cambridge Diagnostic Products Inc., Fort Lau-

derdale, Florida, U.S.A.). In addition, the urinary micro-albumin to creatinine ratio (UAlb/Cre) was measured in each group before and on day 30 after the start of treatment. Urine for the measurement of micro-albumin and creatinine concentration was collected aseptically by a sterilized silicone elastomer-coated Foley catheter and centrifuged at $500 \times g$ for 15 min at 4°C to obtain the supernatant. The samples used for micro-albumin and creatinine concentration were immediately stored at -20°C until assay. Urinary micro-albumin and creatinine were determined by an automatic analyzer (DCA2000, Me-Labo system Co., Ltd., Tochigi, Japan). UAlb/Cre was calculated using the following formula:

$\text{UAlb/Cre (mg/g)} = \text{urinary micro-albumin (mg/l) / urinary creatinine concentration (g/l)}$

Urinary enzyme indices: Urinary enzyme activities, including N-acetyl-beta-D-glucosaminidase (NAG) and GGT were measured in each group before and every day after treatment started. Urine for the measurement of NAG activity, GGT activity, and creatinine concentration was collected aseptically using a sterilized catheter and was then centrifuged at $500 \times g$ for 15 min at 4°C to obtain the supernatant. The samples that were used for NAG activity and creatinine concentration were immediately stored at -20°C , and the samples that were used for GGT activity were immediately stored at 4°C . Measurements were done within a week of collection. Urinary NAG and GGT activities and urinary creatinine concentration were determined by the method reported by Narita *et al.* [30]. Urinary NAG and GGT indices were calculated using the following formulae:

$\text{NAG index (U/g)} = \text{urinary NAG activity (U/l) / urinary creatinine concentration (g/l)}$

$\text{GGT index (U/g)} = \text{urinary GGT activity (U/l) / urinary creatinine concentration (g/l)}$

Hemostatic function tests: Buccal mucosa bleeding time (BMBT) and cuticle bleeding time (CBT) tests were measured as the primary hemostatic function tests, and prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen concentration (FC) were measured as the secondary hemostatic function tests in both groups before and every 7 days after starting the treatment. Before endoscopic examination, by use of a 21-gauge thin-walled needle, 1.8 ml of blood was withdrawn by venipuncture from the jugular vein into a polypropylene syringe containing 0.2 ml of 3.8% trisodium citrate to obtain a final anticoagulant-to-blood ratio of 1:9. Then, blood was immediately centrifuged at $1,800 \times g$ for 15 min at 4°C to obtain the plasma. The samples were immediately supplied to the commercial laboratory (SRL Inc., Tokyo, Japan) and the coagulation panel (PT and APTT) and FC were measured. After endoscopic examination under the anesthetic condition, BMBT and CBT were measured by use of method described by Jergens *et al.* [14] and Giles *et al.* [17], respec-

tively. Briefly, BMBT determinations, a 5-cm-wide strip of gauze was positioned in around the maxilla to fold up the upper lip. The gauze was tied tightly enough to retard venous return, causing moderate engorgement of the mucosal surface. A 1-blade, spring-loaded device (Surgicutt adult, International Technidyne Corporation, NJ, U.S.A.) was used to make one 5-mm (long) and 1-mm (deep) incisions in the upper lip mucosa. Selected incision sites were devoid of visible blood vessels and were inclined so that blood shed after the incision would flow with gravity toward the dog's mouth. At the exact time the incisions were made, a stop watch was started. Shed blood was blotted at approximately 5-s intervals, using a circle of filter paper, which was periodically placed gently against the mucosal surfaces 1 to 2 mm below the incisions; care was taken to prevent direct disturbance of the incision sites. For each incision, bleeding-time end points were recorded at the time the edge of the filter paper did not develop a red crescent when positioned near the incision sites. CBT determinations, silicone grease were applied to the claw in order to prevent blood from tracking back beneath the nail before measuring. Where the apex of the cuticle could be visualized and blood allowed to fall freely by positioning the paw over the edge of the operating table. A commercial nail clipper was used to cut the nail cuticle. Bleeding-time end points were recorded when the spontaneous hemostasis in cut cuticle was observed.

Statistical analyses: Endoscopic lesion grade and fecal occult blood grade data were analyzed by nonparametric statistical methods. The endoscopic lesion grade data was analyzed using Steel-Dwass nonparametric multiple comparison tests to compare differences among treatments at each time-point for each gastrointestinal site. Steel-Dwass nonparametric multiple comparison tests were also used to compare difference among treatments at each time period and within treatments over time for the fecal occult blood grade data analysis. A value of $P < 0.01$ was considered significant for endoscopic lesion grade data due to multiplicity, and a value of $P < 0.05$ was considered significant for fecal occult blood grade data. Data are reported as median, interquartile range (25th to 75th percentile) and 10th percentile point (10th to 90th percentile), or as the median and the range scale.

CBC, blood biochemistry, EPRF, GFR, UAlb/Cre, BMBT, CBT, PT, APTT, and FC were analyzed by parametric statistical methods. These data were analyzed by Tukey multiple comparison tests. For all parametric analyses, a value of $P < 0.05$ was considered significant; data are reported as mean and SD.

RESULTS

Concurrent oral administration was tolerated by all of the dogs, and all dogs completed the study. All dogs in the KP group irregularly showed clinical signs of gastrointestinal disorders, such as anorexia or vomiting, after starting the treatment, and 4 dogs (Nos. 13, 14, 16, and 18) showed diar-

Table 1. Endoscopic lesion grade in gastrointestinal mucosa at pretreatment and during the experiment in each group

	Group	Pretreatment	Day 7	Day 14	Day 21	Day 28
Esophagus	NC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	KP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cardia/fundus	NC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	KP	0 (0)	0 (0 to 1)	1 (0 to 4)	1 (0 to 4)	3 (0 to 4)
Stomach body	NC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MP	0 (0)	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)	0 (0 to 4)
	KP	0 (0)	0 (0 to 1)	0 (0 to 5)	2 (0 to 4)	3 (0 to 6)
Body-antrum junction	NC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MP	0 (0)	0 (0 to 1)	0 (0 to 1)	0 (0)	0 (0)
	KP	0 (0)	0 (0 to 2)	2 (0 to 4)	2 (0 to 5)	3 (2 to 6) ^{†‡}
Angular ventriculi	NC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MP	0 (0)	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)	0 (0)
	KP	0 (0)	0 (0 to 1)	1 (0 to 2)	1 (0 to 2)	0 (0 to 2)
Pyloric antrum	NC	0 (0)	0 (0)	0 (0 to 1)	0 (0 to 2)	0 (0 to 1)
	MP	0 (0)	1 (0 to 1)	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)
	KP	0 (0)	4 (0 to 5)	4 (4 to 5) ^{†‡}	5 (2 to 5) [‡]	5 (4 to 6) ^{†‡}
Pylorus	NC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MP	0 (0)	1 (0 to 2)	0 (0 to 2)	1 (0 to 3)	0 (0 to 1)
	KP	0 (0)	3 (0 to 4)	4 (0 to 4)	4 (0 to 4)	4 (0 to 5)
Duodenum	NC	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	MP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	KP	0 (0)	0 (0)	0 (0)	0 (0 to 2)	0 (0 to 2)

Data were represented as median and range scale.

(): Range scale.

†: $P < 0.05$ compared with negative control group.

‡: $P < 0.05$ compared with meloxicam and prednisolone group.

rhea or melena from day 27 to day 30. However, physical examinations revealed no other abnormalities in any of the groups. Blood analyses exhibited no significant differences between pre- and post-treatment in any of the groups.

Endoscopic examination: Table 1 shows the results of the endoscopic examinations for each group. In the KP group, all 6 dogs had moderate to severe stomach mucosal lesions on day 28; 4 dogs had gastric ulcers in the stomach body (No. 14), body-antrum junction (No. 16), and pyloric antrum (Nos. 13 and 18) with invasive erosions or extensive hemorrhages at other stomach sites; and 2 dogs developed invasive erosions or extensive hemorrhages, especially in the pyloric antrum (Fig. 1). On the other hand, 3 dogs in the NC group and 5 dogs in the MP group developed small erosions in the stomach body, body-antrum junction, angular ventriculi, pyloric antrum, or pylorus during the experimental period, and only 1 dog (No. 9) in the MP group had 3 invasive erosions in the stomach on day 28. At each site of the gastrointestinal tract, no significant differences were observed between the NC and the MP groups. On day 28, the body-antrum junction lesion grade was significantly higher in the KP group on day 28 than in the other groups (each $P = 0.005$). The pyloric antrum lesion grade was also significantly higher in the KP group than in the other groups

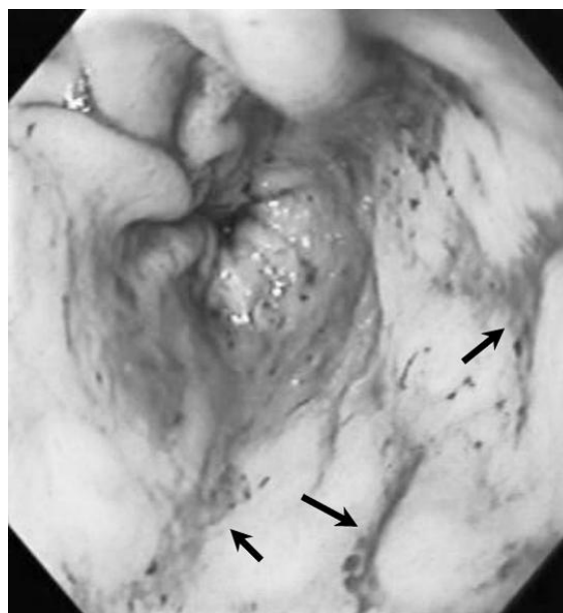


Fig. 1. Endoscopic view of the pyloric antrum on day 28 in a dog treated with the combination of ketoprofen and prednisolone. Notice severe hemorrhage and ulcers (arrows).

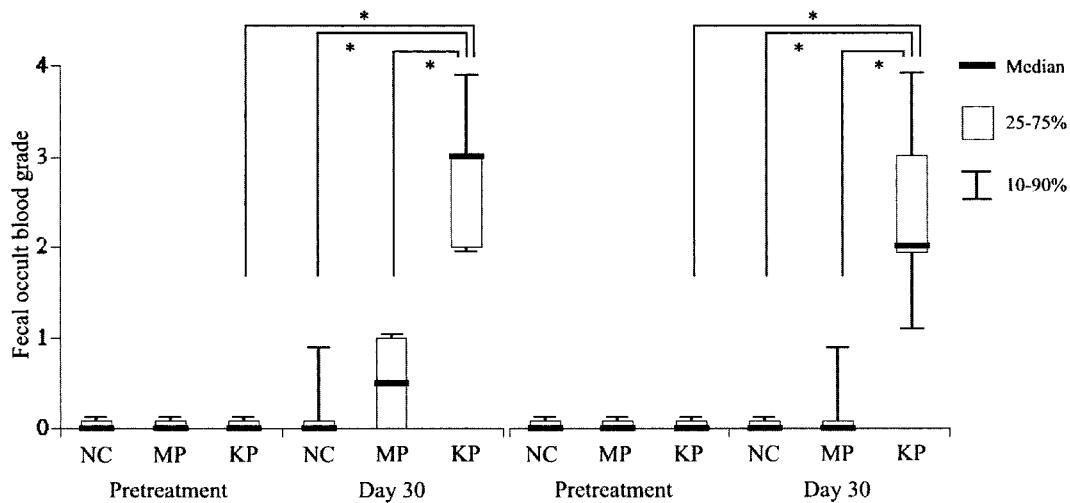


Fig. 2. Fecal occult blood grades determined by use of 2 methods at pretreatment and on day 30 in each group. Box indicates interquartile range, thick line indicates median, and whiskers indicate 10th to 90th percentile. *: Significant differences were observed between pretreatment and on day 30 in the ketoprofen and prednisolone (KP) group, and between the KP and the other groups on day 30 at $P < 0.05$.

on day 14 (each $P = 0.006$) and on day 28 (each $P = 0.007$), and compared to the MP group on day 21 ($P = 0.007$).

Fecal occult blood tests: No dogs had positive fecal occult blood before treatment. However, 6 dogs in the KP group and 1 dog in the MP group had positive fecal occult blood results on day 30, using either testing method. In the KP group, 4 dogs (Nos. 13, 14, 16, and 18) had the highest recorded grades (grade 3 to 4 using both testing methods) on day 30 (Fig. 2). These 4 dogs also had diarrhea or melena on day 30 and had the highest endoscopic lesion grade in the stomach on day 28 (grade 6, a gastric ulcer). In the NC group, 1 dog had grade 1 fecal occult blood using the tetramethylbenzidine method on day 30. In the MP group, 1 dog had grade 1 fecal occult blood using both methods, and 2 dogs had grade 1 fecal occult blood using the tetramethylbenzidine method on day 30. No significant differences were observed in either method between the NC and the MP groups. The fecal occult blood grade determined by both methods was significantly higher in the KP group on day 30, compared with pretreatment (each $P = 0.023$), and compared with either of the other groups on day 30: tetramethylbenzidine method, KP vs. NC group ($P = 0.037$), KP vs. MP group ($P = 0.031$); guaiac method, KP vs. NC group ($P = 0.023$), KP vs. MP group ($P = 0.039$).

Renal function tests: The reference ranges for ERPF and GFR, determined in a preliminary study (18 healthy Beagle dogs), were 12.7 ± 2.3 ml/min/kg and 4.5 ± 1.0 ml/min/kg, respectively. The ERPF and GFR values fell within the reference ranges at all times in the NC and MP groups, and no significant differences were observed in the ERPF and GFR between the NC and the MP groups (Fig. 3). However, in the KP group, the ERPF of 2 dogs (Nos. 13 and 18) was below the reference range on day 10 (7.85 and 8.8 ml/min/kg, respectively), and the ERPF of all dogs was also below

the reference range on day 20 (7.22 to 10.10 ml/min/kg) and on day 30 (6.10 to 9.64 ml/min/kg); the GFR of 5 dogs (Nos. 13, 14, 16, 17 and 18) was below the reference range on day 20 (2.00 to 3.32 ml/min/kg) and on day 30 (1.51 to 2.70 ml/min/kg). The ERPF was significantly lower in the KP group on day 20 and on day 30, compared with pretreatment and compared with the other groups on day 20 and 30 (each $P < 0.001$). The GFR was also significantly lower in the KP group on day 30 compared with pretreatment ($P < 0.001$), and on day 20 and on day 30 compared with the other groups; day 20, KP vs. NC group ($P < 0.001$), KP vs. MP group ($P = 0.021$); day 30; KP vs. NC group ($P < 0.001$), KP vs. MP group ($P < 0.001$).

Urinalyses: In the NC and MP groups, the dipstick test and the urine specific gravity showed few changes over the sampling period. However, all dogs in the KP group showed mild to moderate proteinuria (100 to 300 mg/dl) on days 20, 25, and 30. According to the manufacturer's instructions, the reference range of the UAlb/Cre in healthy dogs is 13.1 ± 15.6 mg/g. The UAlb/Cre in all groups at pretreatment and in the NC and MP groups on day 30 was within the reference range. However, in the KP group, the UAlb/Cre of 5 dogs (Nos. 13, 14, 16, 17 and 18) was above the reference range on day 30 (31.2 to 156 mg/g). No significant differences were observed in the UAlb/Cre between the NC and the MP groups. However, the UAlb/Cre was significantly higher in the KP group on day 30, both compared to the pretreatment value and compared to the other groups on day 30 (each $P < 0.001$) (Fig. 4).

Urinary enzyme indices: Based on previous studies, the reference ranges in dogs for the NAG and GGT indexes were 3.2 ± 2.4 U/g and 31.6 ± 10.4 U/g, respectively [30, 36]. In the NC group, the urinary enzyme indices, both NAG and GGT, were almost within the reference range;

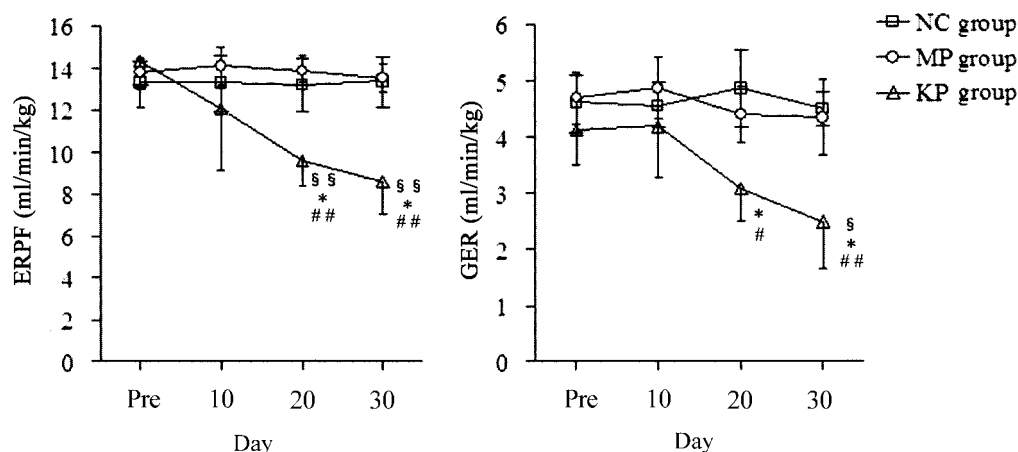


Fig. 3. Changes in effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) before treatment (Pre) and during the experimental period (day 10 to 30) for each group. Each symbol and bar represents mean and SD, respectively. §, §§: Significant difference from the pretreatment value at $P<0.01$, $P<0.001$, respectively. *: Significant difference from the negative control (NC) group at $P<0.001$. #, ##: Significant difference from the meloxicam and prednisolone (MP) group at $P<0.05$, $P<0.001$, respectively.

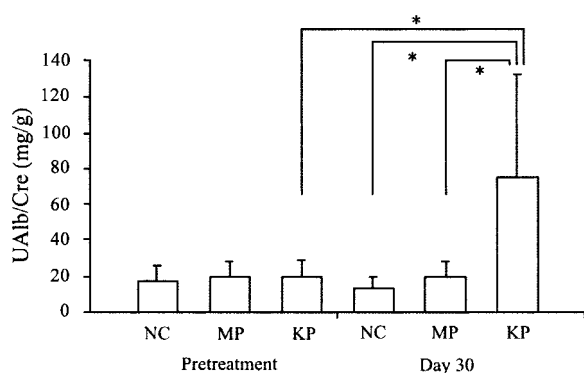


Fig. 4. Urinary micro-albumin to creatinine ratio (UAlb/Cre) at pretreatment and on day 30 for each group. Each column and bar represents mean and SD, respectively. *: A significant difference was observed between the pretreatment value and the day 30 value in the KP group, and between the KP and the other groups on day 30 at $P<0.001$.

however, in the KP and MP groups, irregularly abnormal enzymuria was observed alternating from hyper- to hypo-enzymuria in both urinary enzyme indices, which correlated with the number of RTE cells in the urine (1 to 5 RTE cells / HPF were observed at 5 to 30 days) (Fig. 5).

Hemostatic function tests: In the NC and MP groups, the BMBT and CBT were within the reference ranges at all times; no significant differences were detected in either parameter. However, in the KP groups, 1 dog had a prolonged BMBT on day 14 (No. 17; 4.47 min), 2 dogs had a prolonged BMBT on day 21 (Nos. 17 and 18; 4.78 min and 4.89 min, respectively) and 3 dogs had a prolonged BMBT on day 28 (Nos. 16, 17, and 18; 5.78 to 9.72 min); 2 dogs had a prolonged CBT on day 21 (Nos. 17 and 18; 9 and 15 min, respectively) and 3 dogs had a prolonged CBT on day

28 (Nos. 16, 17 and 18; 18 to 30 min). BMBT was significantly prolonged in the KP groups on day 28, compared to the pretreatment value ($P=0.002$) and compared to the other groups on day 28 (KP vs. NC group [$P=0.013$], KP vs. MP group [$P=0.014$]). As well, the CBT was significantly prolonged in the KP group on day 28, compared to the pretreatment value and to the other groups on day 28 (each $P<0.001$) (Fig. 6). Based on a previous study, the reference ranges were: PT, 5.8 to 7.9 seconds; APTT, 11.6 to 18.3 seconds; and for FC, 160 to 254.5 mg/dl [31]. The PT, APTT, and FC values were within these reference ranges in all groups at all times, and no significant differences were detected in these parameters among the groups (Table 2).

DISCUSSION

In the present study, we demonstrated that NSAIDs co-administered with prednisolone induced considerable adverse effects on the kidneys, platelet function, and the gastrointestinal mucosa. To the best of our knowledge, this study is the first to analyze the adverse effects on renal function, enzymuria, albuminuria, and bleeding time of combination treatment with NSAIDs and prednisolone in healthy dogs.

With respect to the gastrointestinal mucosa, there were significant numbers of severe gastric mucosal injuries, in particular gastric ulcers, and strongly positive fecal occult bloods, which are reported to be useful to diagnose NSAID-induced gastric mucosal injury [30, 31]. These findings were observed in the dogs given a combination of ketoprofen and prednisolone; these dogs also showed clinical signs such as anorexia, vomiting, diarrhea, or melena. The co-administration of NSAIDs and corticosteroids has been well known to produce severe gastric mucosal adverse effects such as gastric ulcer or perforation. Dow *et al.* reported that

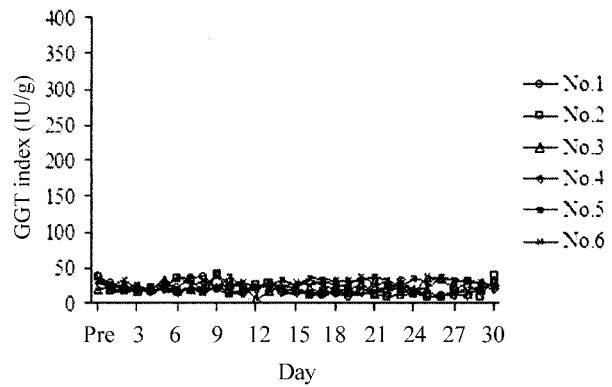
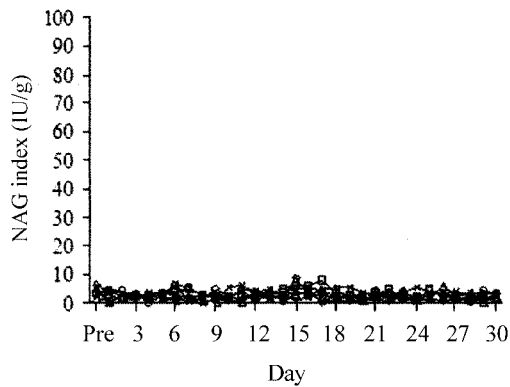
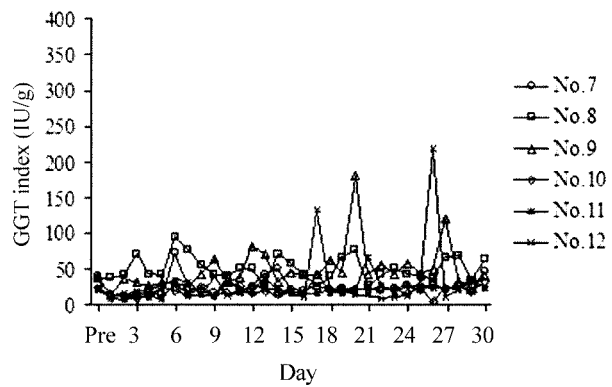
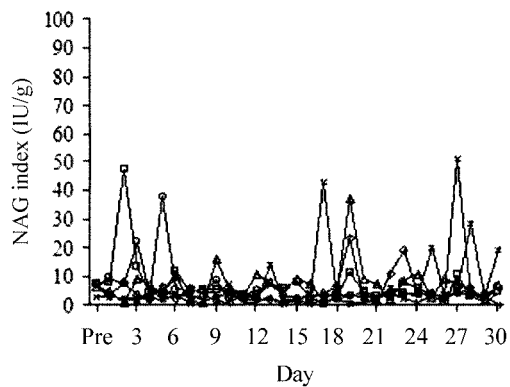
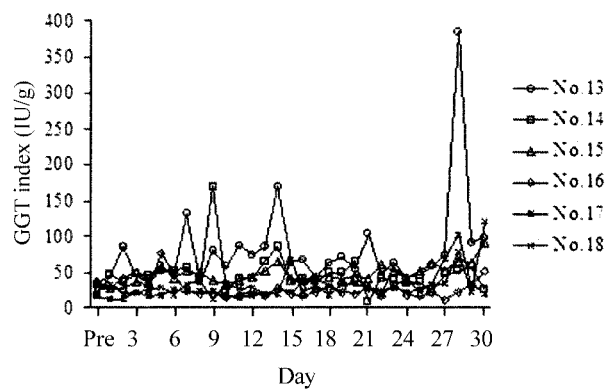
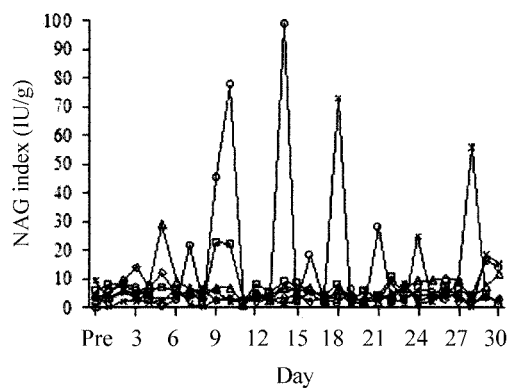
NC group**MP group****KP group**

Fig. 5. Changes in the NAG and the GGT indices before treatment (Pre) and during the experimental period (days 1 to 30) for each group. Each symbol and bar represents the mean and SD, respectively. Abnormal enzymuria, fluctuating between hyper- and hypo-enzymuria, was observed in the MP and KP groups.

flunixin plus prednisone exacerbated the gastrointestinal injury induced by flunixin alone [6]. As well, 29 cases of gastrointestinal tract perforation were found to be associated with the use of deracoxib, a specific COX-2 inhibitor that is

supposed to have a good safety profile; 3 of 29 dogs had received corticosteroids before, after, or concurrently with deracoxib treatment [24]. In contrast to the effects seen with prednisolone, dexamethasone markedly reduced the extent

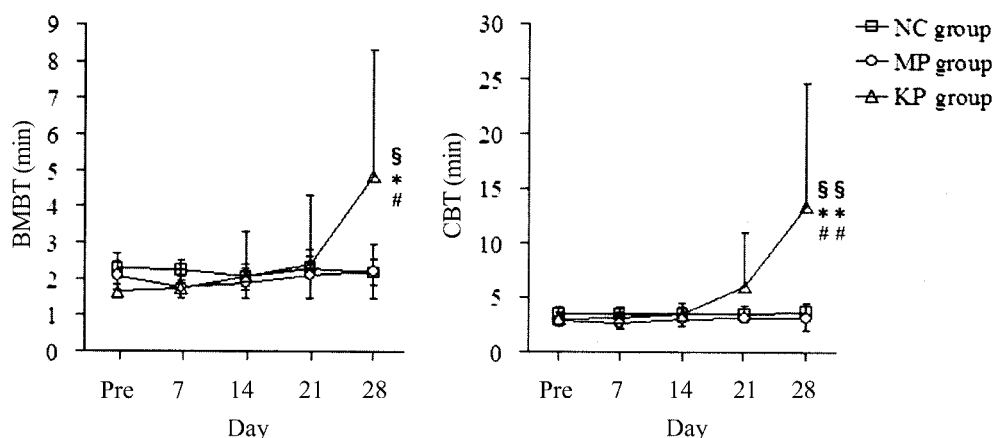


Fig. 6. Changes in the buccal mucosa bleeding time (BMBT) and the cuticle bleeding time (CBT) before treatment (Pre) and during the experimental period (days 7 to 28) for each group. Each symbol and bar represents mean and SD, respectively. §, §§: Significant difference from the pretreatment value at $P < 0.01$, $P < 0.001$, respectively. *, **: Significant difference from the NC group at $P < 0.05$, $P < 0.001$, respectively. #, ##: Significant difference from the MP group at $P < 0.05$, $P < 0.001$, respectively.

Table 2. Results of measurements of prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen concentration (FC) at the pretreatment and during the experiment in each group

Variable	Group	Pretreatment	Day 7	Day 14	Day 21	Day 28
PT (sec)	NC	6.63 ± 0.21	6.45 ± 0.89	7.38 ± 0.74	6.28 ± 0.77	6.48 ± 0.96
	MP	6.92 ± 0.53	7.12 ± 0.58	7.15 ± 0.42	6.98 ± 0.69	7.08 ± 1.13
	KP	6.62 ± 0.32	6.93 ± 0.46	6.62 ± 0.34	7.12 ± 0.32	6.62 ± 0.48
APTT (sec)	NC	13.7 ± 1.78	15.6 ± 2.05	15.8 ± 2.43	14.7 ± 2.56	15.9 ± 1.99
	MP	15.4 ± 2.61	15.6 ± 1.77	16.3 ± 2.08	16.2 ± 1.93	15.4 ± 0.73
	KP	13.2 ± 1.30	13.5 ± 1.29	14.4 ± 1.29	13.9 ± 0.89	14.6 ± 1.30
FC (mg/dl)	NC	203.3 ± 52.7	234.3 ± 68.5	222.5 ± 48.4	207.8 ± 3.63	232.2 ± 43.1
	MP	233.2 ± 58.4	256.2 ± 37.1	231.2 ± 35.7	226.0 ± 2.85	227.7 ± 34.4
	KP	205.5 ± 46.4	203.3 ± 22.9	195.8 ± 16.1	191.7 ± 21.0	230.8 ± 33.2

Data are reported as mean ± SD.

of gastric damage induced by indomethacin via the inhibition of intercellular adhesion molecule-1 and leukocyte adherence in arthritic rats [28]. Kataoka *et al.* reported that the combination of nimesulide and prednisolone produced no gastric lesions in rat stomachs [19], and Forsyth *et al.* reported that oral cinchophen and prednisolone given concurrently for 7 days caused no significant gastric mucosal injury in dogs [9]. However, there are some controversial results dealing with the safety of combined NSAIDs and corticosteroids in animals with gastric mucosal injury. The dosage of ketoprofen used in the present study was 0.25 mg/kg; we have previously reported that ketoprofen alone at a reduced dosage had caused mild to moderate gastric mucosal injuries, such as invasive erosions, without clinical signs in healthy dogs [31]. Tanaka *et al.* have also reported that the gastric ulcerogenic property of NSAIDs cannot be accounted for solely by COX-1 inhibition; it requires the inhibition of both COX-1 and COX-2 [39].

One dog in the NC group exhibited the weakly positive fecal blood, and had small mucosal erosion in the pyloric

antrum. The cause of gastric mucosal erosion and fecal occult blood in the NC group was unknown, however, it is similar to finding of our previous study in which gastric mucosal injuries and weakly fecal occult blood were detected in the small number of the dogs in gelatin placebo group [30, 31], and other study [10] in which a small number of pinpoint gastric haemorrhages were observed in the gelatin group. Therefore, we speculate that fecal occult blood and gastric injuries in the NC group may be caused by several stressors such as capsule administration or anesthesia.

Corticosteroids can affect PG synthesis in several tissues, via a strong blockade at the level of mRNA transcription for COX-2 [8, 29] and a weak blockade at the level of mRNA transcription for COX-1 [38], and the inhibition of phospholipase A₂ [2] that synthesizes arachidonic acid from phospholipid [18]. Therefore, corticosteroids, even a low dosage of prednisolone (0.5 mg/kg), are thought to aggravate gastric mucosal adverse effects of reduced dosage ketoprofen (0.25 mg/kg) via the blockade of COX-2 expression and the

inhibition of phospholipase A₂. Although the group given the combination of meloxicam and prednisolone developed a few invasive erosions, with only 1 dog having the worst lesions, no significant difference was observed between the dogs in the MP group and the gelatin group. Meloxicam is a selective COX-2 inhibitor, and the safety of meloxicam use in dogs has been previously described [10]. In the present study, there were fewer gastric mucosal adverse effects of combined meloxicam and prednisolone than in a previous study, in which the short-term concurrent administration of meloxicam (0.1 mg/kg, PO, q 24 hr) and dexamethasone (0.25 mg/kg, SC, q 12 hr) for 3 days caused more gastric erosions, but not gastric ulcers, than meloxicam alone [4]. Dexamethasone is thought to have more ulcerogenic power than prednisolone, and some reports have shown that a non-ulcerogenic dose of dexamethasone delayed gastric ulcer healing in animals [26, 27]. The exact reason for this is still unclear. However, the difference in the findings among the studies may be due to the different corticosteroids used in each study. Therefore, our results indicate that meloxicam is safer than ketoprofen with respect to the gastrointestinal tract, even when administered concomitantly with prednisolone, though this in no way suggests that the combination of meloxicam and prednisolone is safe for clinical use.

With respect to the kidneys, the combination of ketoprofen and prednisolone caused a significant decrease in renal function, defined as a decrease of RPF and GFR, and hyperalbuminuria. A pronounced abnormal enzymuria associated with exfoliation of RTE cells in the urine was also observed. Little appears to be known about the aggravation of renal adverse effects that occurs when NSAIDs and corticosteroids are given concurrently in dogs. We have previously reported that RPF and GFR fell within the reference ranges and that there was no elevation of the NAG and GGT indices in healthy dogs treated with a reduced dosage of ketoprofen alone [31]. Kay-Mugford *et al.* reported that Madin-Darby canine kidney cells expressed both COX-1 and COX-2 [21]. Furthermore, COX-2 is sparsely expressed in the macula densa of the normal dog kidney, and up-regulation of COX-2 in the kidney and the stomach [16] has also been reported by several studies in dogs with volume or salt depletion; these results suggest that COX-2 and COX-1 have a possible role in the response that helps maintain renal function [3, 22, 40]. Thus, it is thought that ketoprofen inhibits mainly COX-1 in the kidney and that prednisolone may concomitantly block up-regulation of COX-2 that helps maintain renal function, which results in a decrease of RPF and GFR and causes the renal tubular injuries and hyperalbuminuria, which are early marker of renal disease in dogs [12, 13, 25]. On the other hand, fairly abnormal enzymuria with exfoliation of RTE cells in the urine was observed, although meloxicam co-administered with prednisolone showed no other significant changes in renal function and albuminuria compared with the negative control group and the pretreatment values. The reason that these changes occurred without a decrease in renal function is not known;

they may be due to acute interstitial nephritis (AIN) or acute tubular necrosis (ATN). In humans, AIN and ATN have been described in association with a specific COX-2 inhibitor [1, 7], but, to our knowledge, AIN or ATN associated with meloxicam has not been reported in veterinary medicine. Thus, further study is needed to evaluate the abnormal enzymuria and exfoliation of RTE cells to determine whether this occurs as a result of meloxicam alone or as a result of concurrent administration of meloxicam and prednisolone.

With respect to platelet function, the combination of meloxicam and prednisolone resulted in no significant changes in bleeding times, whereas the combination of ketoprofen and prednisolone caused prolonged bleeding times using both methods, without affecting measures of secondary hemostasis. The buccal mucosa bleeding time and the cuticle bleeding time were the bleeding tests used in the present study to assess several aspects of primary platelet plug formation, and have been shown to be the best *in vivo* tests of primary hemostasis [14, 17]. We have previously reported that the long-term administration of ketoprofen (0.25 mg/kg and 1.0 mg/kg) has no effect on bleeding times in dogs [30, 31], and Frenso *et al.* reported that the short-term use of meloxicam caused no significant changes in dog hemostatic variables [11]. Platelets are the only cells that exclusively express COX-1 in dogs [21]. COX-1 forms thromboxane A₂, which in turn produces primary platelet plugs, through endogenous arachidonic acid that is supplied by phospholipase A₂. Thus, ketoprofen and prednisolone may prevent COX-1 and phospholipase A₂ from acting, which would result in platelet aggregation failure and prolonged bleeding times. However, the dogs that received ketoprofen and prednisolone exhibited no clinical signs of platelet dysfunction, such as petechiae or ecchymoses. Therefore, clinicians should be aware of the potential risk of hemorrhage during surgery in dogs that have been given NSAIDs, such as ketoprofen, concurrently with prednisolone, even if there are no clinical sign of abnormal hemostasis.

In conclusion, the combination of ketoprofen and prednisolone caused severe adverse effects on the kidneys, platelet function, and the gastrointestinal mucosa. On the other hand, meloxicam and prednisolone given concurrently caused fairly abnormal enzymuria with exfoliation of RTE cells in the urine, which may be due to acute interstitial nephritis (AIN) or acute tubular necrosis (ATN). Thus, even in healthy dogs, the concurrent administration of NSAIDs and corticosteroids may be contraindicated, even if the NSAID is a selective COX-2 inhibitor that has an excellent safety profile with respect to the gastrointestinal tract. Further study is needed to evaluate the adverse renal effects of meloxicam or prednisolone alone.

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REFERENCES

- Almansori, M., Kovithavongs, T. and Qarni, M. U. 2005. Cyclooxygenase-2 inhibitor-associated minimal change disease. *Clin. Nephrol.* **63**: 381–384.
- Barnes, P. J. and Adcock, I. 1993. Anti-inflammatory actions of steroids: molecular mechanisms. *Trends. Pharmacol. Sci.* **14**: 436–441.
- Black, S. C., Brideau, C., Cirino, M., Belley, M., Bosquet, J., Chan, C. C. and Rodger, I. W. 1998. Differential effect of a selective cyclooxygenase-2 inhibitor versus indomethacin on renal blood flow in conscious volume-depleted dogs. *J. Cardiovasc. Pharmacol.* **32**: 686–694.
- Boston, S. E., Moens, N. M. M., Kruth, S. A. and Southorn, E. P. 2003. Endoscopic evaluation of the gastroduodenal mucosa to determine the safety of short-term concurrent administration of meloxicam and dexamethasone in healthy dogs. *Am. J. Vet. Res.* **64**: 1369–1375.
- Deneuche, A. J., Dufayet, C., Goby, L., Fayolle, P. and Desbois, C. 2004. Analgesic comparison of meloxicam or ketoprofen for orthopedic surgery in dogs. *Vet. Surg.* **33**: 650–660.
- Dow, S. W., Rosychuk, R. A. W., McChesney, A. E. and Curtis, C. R. 1990. Effects of flunixin and flunixin plus prednisone on the gastrointestinal tract of dogs. *Am. J. Vet. Res.* **51**: 1131–1138.
- Esteve, J. B., Launay-Vacher, V., Brocheriou, I., Grimaldi, A. and Izzedine, H. 2005. COX-2 inhibitors and acute interstitial nephritis: case report and review of the literature. *Clin. Nephrol.* **63**: 385–389.
- Evett, G. E., Xie, W., Chipman, J. G., Robertson, D. L. and Simmons, D. L. 1993. Prostaglandin G/H synthase isoenzyme 2 expression in fibroblast: regulation by dexamethasone, mitogens, and oncogenes. *Arch. Biochem. Biophys.* **306**: 169–177.
- Forsyth, S. F., Guilford, W. G. and Lawoko, C. R. O. 1996. Endoscopic evaluation of the gastroduodenal mucosa following non-steroidal anti-inflammatory drug administration in the dog. *N. Z. Vet. J.* **44**: 179–181.
- Forsyth, S. F., Guilford, W. G., Haslett, S. J. and Godfrey, J. 1998. Endoscopy of the gastroduodenal mucosa after carprofen, meloxicam and ketoprofen administration in dogs. *J. Small Anim. Pract.* **39**: 421–424.
- Frenso, L., Moll, J., Penalba, B., Espada, Y., Andaluz, A., Prandi, D., Ruiz de Gopegui, R. and Garcia, F. 2005. Effects of preoperative administration of meloxicam on whole blood platelet aggregation, buccal mucosal bleeding time, and haematological indices in dogs undergoing elective ovariohysterectomy. *Vet. J.* **170**: 138–140.
- Gary, A. T., Cohn, L. A., Kerl, M. E. and Jensen, W. A. 2004. The effects of exercise on urinary albumin excretion in dogs. *J. Vet. Intern. Med.* **18**: 52–55.
- Gentilini, F., Dondi, F., Mastroilli, C., Giunti, M., Calzolari, C., Gandini, G., Mancini, D. and Bergamini, P. F. 2005. Validation of a human immunoturbidimetric assay to measure canine albumin in urine and cerebrospinal fluid. *J. Vet. Diagn. Invest.* **17**: 179–183.
- Giles, A. R., Tinlin, S. and Greenwood, R. 1982. A canine model of hemophilic (Factor VIII: C deficiency) bleeding. *Blood.* **60**: 727–730.
- Hazewinkel, H. A. W., van den Brom, W. E., Theijse, L. F. H., Pollmeier, M. and Hanson, P. D. 2003. Reduced dosage of ketoprofen for the short-term and long-term treatment of joint pain in dogs. *Vet. Rec.* **152**: 11–14.
- Jackson, L. M., Wu, K. C., Mahida, Y. R., Jenkins, D. and Hawkey, C. J. 2000. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. *Gut.* **47**: 762–770.
- Jergens, A. E., Turrentine, M. A., Kraus, K. H. and Johnson, G. S. 1987. Buccal mucosa bleeding times of healthy dogs and of dogs in various pathologic states, including thrombocytopenia, uremia, and von Willebrand's disease. *Am. J. Vet. Res.* **48**: 1337–1342.
- Johnston, S. A. and Budsberg, S. C. 1997. Nonsteroidal anti-inflammatory drugs and corticosteroids for the management of canine osteoarthritis. *Vet. Clin. North. Am. Small. Anim. Pract.* **27**: 841–862.
- Kataoka, H., Horie, Y., Koyama, R., Nakatsugi, S. and Furukawa, M. 2000. Interaction between NSAIDs and steroid in rat stomach. *Dig. Dis. Sci.* **45**: 1366–1375.
- Kay-Mugford, P., Benn, S. J., LaMarre, J. and Conlon, P. 2000. *In vitro* effects of nonsteroidal anti-inflammatory drugs on cyclooxygenase activity in dogs. *Am. J. Vet. Res.* **61**: 802–810.
- Kay-Mugford, P., Benn, S. J., LaMarre, J. and Conlon, P. 2000. Cyclooxygenase expression in canine platelets and Madin-Darby canine kidney cells. *Am. J. Vet. Res.* **61**: 1512–1516.
- Khan, K. N., Venturini, C. M., Bunch, R. T., Brassard, J. A., Koki, A. T., Morris, D. L., Trump, B. F., Maziasz, T. J. and Alden, C. L. 1998. Interspecies differences in renal localization of cyclooxygenase isoforms: implications in nonsteroidal anti-inflammatory drug-related nephrotoxicity. *Toxicol. Pathol.* **26**: 612–620.
- Lascelles, B. D. X., McFarland, M. and Swann, H. 2005. Guidelines for safe and effective use of NSAIDs in dogs. *Vet. Ther.* **6**: 237–251.
- Lascelles, B. D. X., Blikslager, A. T., Fox, S. M. and Reece, D. 2005. Gastrointestinal tract perforation in dogs treated with a selective cyclooxygenase-2 inhibitor: 29 cases (2002–2003). *J. Am. Vet. Med. Assoc.* **227**: 1112–1117.
- Lees, G. E., Brown, S. A., Elliott, J., Grauer, G. F. and Vaden, S. L. 2005. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM forum consensus statement (small animal). *J. Vet. Intern. Med.* **19**: 377–385.
- Luo, J. C., Shin, V. Y., Liu, E. S. L., So, W. H. L., Ye, Y. N., Chang, F. Y. and Cho, C. H. 2003. Non-ulcerogenic dose of dexamethasone delays gastric ulcer healing in rats. *J. Pharmacol. Exp. Ther.* **307**: 692–698.
- Luo, J. C., Shin, V. Y., Liu, E. S. L., Ye, Y. N., Wu, W. K. K., So, W. H. L., Chang, F. Y. and Cho, C. H. 2004. Dexamethasone delays ulcer healing by inhibition of angiogenesis in rat stomachs. *Eur. J. Pharmacol.* **485**: 275–281.
- McCafferty, D. M., Grand, D. N. and Wallace, J. L. 1995. Indomethacin-induced gastric injury and leukocyte adherence in arthritic versus healthy rats. *Gastroenterology* **109**: 1173–1180.
- McEwan, I. J., Wright, A. P. and Gustafsson, J. A. 1997. Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. *Bioessays* **19**: 153–160.
- Narita, T., Tomizawa, N., Sato, R., Goryo, M. and Hara, S. 2005. Effects of long-term oral administration of ketoprofen in clinically healthy beagle dogs. *J. Vet. Med. Sci.* **67**: 847–853.
- Narita, T., Sato, R., Tomizawa, N., Tani, K., Komori, S. and Hara, S. 2006. Safety of reduced-dosage ketoprofen for long-term oral administration in healthy dogs. *Am. J. Vet. Res.* **67**: 1115–1120.
- Peterson, K. D. and Keefe, T. J. 2004. Effects of meloxicam on severity of lameness and other clinical signs of osteoarthritis in dogs. *J. Am. Vet. Med. Assoc.* **225**: 1056–1060.

33. Ricketts, A. P., Lundy, K. M. and Seibel, S. B. 1998. Evaluation of selective inhibition of canine cyclooxygenase 1 and 2 by carprofen and other nonsteroidal anti-inflammatory drugs. *Am. J. Vet. Res.* **59**: 1441–1446.
34. Rohrer, C. R., Hill, R. C., Fischer, A., Fox, L. E., Schaer, M., Ginn, P. E., Casanova, J. M. and Burrows, C. F. 1999. Gastric hemorrhage in dogs given high doses of methylprednisolone sodium succinate. *Am. J. Vet. Res.* **60**: 977–981.
35. Rohrer, C. R., Hill, R. C., Fischer, A., Fox, L. E., Schaer, M., Ginn, P. E., Preast, V. A. and Burrows, C. F. 1999. Efficacy of misoprostol in prevention of gastric hemorrhage in dogs treated with high doses of methylprednisolone sodium succinate. *Am. J. Vet. Res.* **60**: 982–985.
36. Sato, R., Soeta, S., Miyazaki, M., Syuto, B., Sato, J., Miyake, Y., Yasuda, J., Okada, K. and Naito, Y. 2002. Clinical availability of urinary N-acetyl-beta-D-glucosaminidase index in dogs with urinary diseases. *J. Vet. Med. Sci.* **64**: 361–365.
37. Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J. and Isakson, P. 1997. Distribution of COX-1 and COX-2 in normal and inflamed tissues. *Adv. Exp. Med. Biol.* **400A**: 167–170.
38. Takahashi, Y., Taketani, Y., Endo, T., Yamamoto, S. and Kumegawa, M. 1994. Studies on the induction of cyclooxygenase isoenzymes by various prostaglandins in mouse osteoblastic cell line with reference to signal transduction pathways. *Biochem. Biophys. Acta* **1212**: 217–224.
39. Tanaka, A., Araki, H., Komoike, Y., Hase, S. and Takeuchi, K. 2001. Inhibition of both COX-1 and COX-2 is required for development of gastric damage in response to nonsteroidal antiinflammatory drugs. *J. Physiol. (Paris)* **95**: 21–27.
40. Venturini, C. M., Isakson, P. and Needleman, P. 1998. Nonsteroidal anti-inflammatory drug-induced renal failure: a brief review of the role of cyclo-oxygenase isoforms. *Curr. Opin. Nephrol. Hypertens.* **7**: 79–82.
41. Wilson, J. E., Chandrasekharan, N. V., Westover, K. D., Eager, K. B. and Simmons, D. L. 2004. Determination of expression of cyclooxygenase-1 and -2 isozymes in canine tissues and their differential sensitivity to nonsteroidal anti-inflammatory drugs. *Am. J. Vet. Res.* **65**: 810–818.