

Systemic absorption and gastrointestinal adverse effects from topical ketorolac and diclofenac ophthalmic solutions in healthy dogs

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OBJECTIVE

To investigate systemic absorption and gastrointestinal (GI) adverse effects of topical ketorolac 0.5% and diclofenac 0.1% ophthalmic solutions.

ANIMALS

11 healthy purpose-bred Beagles.

METHODS

Dogs were randomly assigned to receive either ketorolac ($n = 6$) or diclofenac (5), 1 drop in both eyes 4 times daily for 28 days. Upper GI endoscopy was performed on days 0 and 29 with mucosal lesion scores (0 to 7) assigned to each region evaluated. Plasma samples were collected on days 14, 21, and 28 for measurement of diclofenac and ketorolac using high-performance liquid chromatography–mass spectrometry.

RESULTS

GI erosions and/or ulcers developed in all ketorolac-treated dogs and 1 of 5 diclofenac-treated dogs. Post-treatment mucosal lesion score for the antrum was higher in the ketorolac group than in the diclofenac group ($P = .006$) but not significantly different for any other region. Post-treatment antral mucosal lesion scores were significantly related to plasma ketorolac concentrations ($P < .001$). Ketorolac and diclofenac were detected in the plasma at all time points (median ketorolac day 14, 191 ng/mL; day 21, 173.5 ng/mL; and day 28, 179.5 ng/mL; and median diclofenac day 14, 21.1 ng/mL; day 21, 20.6 ng/mL; day 28, 27.5 ng/mL). Vomiting and decreased appetite events were observed uncommonly and were not significantly different between treatment groups.

CLINICAL RELEVANCE

GI ulceration and erosion developed after ophthalmic administration of ketorolac and diclofenac, with higher plasma concentrations and more severe GI lesions associated with ketorolac. Clients should be alerted to this potential risk with ophthalmic use and informed to watch for systemic clinical signs that would warrant veterinary reevaluation.

Keywords: NSAID, nonsteroidal anti-inflammatory drug, gastrointestinal, systemic, ocular

Gastrointestinal (GI) effects of systemic NSAIDs are well recognized. The mechanism by which NSAIDs damage the gastric mucosal barrier is multifactorial but thought to be primarily via inhibition of prostaglandin synthesis.^{1,2} Prostaglandins function to maintain the integrity of the GI tract by inhibiting gastric acid secretion, stimulating mucus and bicarbonate secretion, and increasing mucosal blood flow, among other protective functions.¹

Topical ophthalmic administration of NSAIDs is routinely used to reduce inflammation in the eye, and ophthalmic NSAIDs are an integral component of managing ocular pain and prevention or management of uveitis in dogs.^{3,4} Because the systemic absorption of these medications is assumed to be clinically insignificant, they are often used in combination with either an oral NSAID or glucocorticoid for more effective treatment of uveitis. Additionally, some dogs receiving topical NSAIDs may be older/geriatric (eg, for the treatment of senile cataracts) and consequentially affected by ≥ 1 comorbid condition that can increase risk of GI bleeding.

The possibility of systemic absorption is known with ocular administration of drugs.⁵ Drugs applied

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in the form of drops to the surface of the eye may be given frequently and at high concentrations, knowing that much of the drop will be lost when diluted with tears and cleared from the ocular surface, with only a small fraction of topical drugs instilled via eye drops available for ocular absorption.⁵ Systemic absorption of these drugs can occur via conjunctival vessel absorption or nasolacrimal drainage, where the drug is subsequently swallowed or absorbed by the nasal mucosa.^{5,6}

Some of the most common ophthalmic NSAID preparations currently used in veterinary medicine are 0.1% diclofenac sodium solution and 0.5% ketorolac tromethamine solution. A recent retrospective study⁷ found similar GI bleeding frequencies in dogs treated with ophthalmic NSAIDs when compared to dogs receiving systemic NSAIDs or systemic glucocorticoids. In this study, severe or fatal outcomes occurred only in the ketorolac-treated dogs.⁷ Systemically administered ketorolac has been shown in humans to be associated with a significantly higher risk of GI ulceration than most other NSAIDs, including diclofenac.⁸⁻¹² Research on the use of parenteral or oral ketorolac is limited in dogs, but it has been shown to be an effective analgesic and has been associated with the development of GI ulcers.¹³⁻¹⁶ Systemic absorption of diclofenac has been documented after ophthalmic administration in cats,^{17,18} but there are no prospective studies evaluating systemic absorption or possible systemic adverse effects for either of these drugs in dogs.

In this study, our aim was to investigate the relationship between systemic exposure to NSAIDs after topical dosing and the development of GI lesions. Specifically, our objectives were to (1) quantify plasma concentrations of diclofenac and ketorolac around their expected peak in healthy Beagles during and after 28 days of ophthalmic administration of diclofenac or ketorolac and (2) to evaluate for GI adverse effects associated with administration of topical NSAIDs. We hypothesized that healthy dogs administered topical diclofenac and ketorolac would have detectable plasma levels of these drugs and develop GI lesions, with more severe lesions associated with the administration of ketorolac.

Methods

Animals

Eleven healthy 2-year-old Beagles from a research colony were used for this study. The study protocol was reviewed and approved by the Iowa State University Animal Care and Use Committee (IACUC 20-196). At baseline, all Beagles had an ophthalmic evaluation including Schirmer tear test, fluorescein staining of the ocular surface, slit lamp biomicroscopy, rebound tonometry, and indirect ophthalmoscopy. Ophthalmic examination and physical examination were normal in all dogs prior to study inclusion. Dogs were housed in pairs in a temperature-controlled (21 °C) facility with a 12-hour light-dark schedule. The dogs had been housed

in this facility for the past year since arriving from the same supplier and had no prior health problems. The pair-housing arrangements that were in place prior to the study were kept consistent throughout the study. The commercially available maintenance diet that the dogs had been receiving prior to the start of the study was fed once a day consistently throughout the study duration to meet daily energy requirements, and water was available ad libitum.

Ophthalmic NSAID treatment

Dogs were randomly assigned to 2 treatment groups using the “psych” and “randomizr” packages in R version 4.3.1 (The R Project for Statistical Computing). Dogs were allocated to treatment group by pen (pair-housed) so both dogs in a pen would receive the same treatment. One treatment group (n = 5 dogs) was assigned to diclofenac sodium (0.1%) ophthalmic drops. The other treatment group (n = 6 dogs) was assigned to ketorolac tromethamine (0.5%) ophthalmic drops. Since this was a colony of 11 dogs, 1 dog in the diclofenac group was housed singly throughout the study. One drop of diclofenac or ketorolac was applied to both eyes 4 times a day (approx 8 AM, 12 PM, 4 PM, and 8 PM) for 28 days. All investigators were masked to treatment group allocation throughout the duration of the study.

Fecal occult blood testing

Voided feces from each pen were collected for fecal occult blood evaluation on days 0, 14, 21, and 28 using a guaiac paper test (Hemoccult; Beckman Coulter). An applicator stick was used to sample the feces in 2 different sites prior to applying to the guaiac slide. The tests were developed between 3 and 14 days after sample collection as recommended by the manufacturer and any blue color was interpreted as a positive result.¹⁹

Gastroduodenoscopy

Gastroduodenoscopy was performed in all dogs on days 0 and 29. Dogs were anesthetized with butorphanol, acepromazine, and propofol and placed in left lateral recumbency. Endoscopic examination was performed in the same order for all dogs by the same investigator (LRV), as previously described.²⁰ Briefly, examination of the esophagus, gastric body, body/antrum junction, antrum, and retroflexed examination of the fundus/cardia were performed followed by evaluation of the duodenum. All sites were examined sequentially and assigned a mucosal score prior to moving on to the next location. The mucosal score was adapted from endoscopic lesion scoring systems as previously described. Each site was scored from 0 to 7, with 0, no visible lesions; 1, 1 to 10 petechiae; 2, > 10 petechiae; 3, 1 to 5 erosions (break in the mucosa); 4, 6 to 10 erosions; 5, 11 to 25 erosions; 6, > 25 erosions; and 7, ulcer (a wide break in the mucosa with a craterous appearance).^{20,21}

Monitoring

Dogs were observed 4 times daily at the time of eye drops administration. The presence of any vomit

was recorded by pen. Feces were observed by pen (pair) once daily and assigned a fecal score by use of the Purina 7-point fecal scoring system (<https://www.purinainstitute.com/centresquare/nutritional-and-clinical-assessment/purina-fecal-scoring-chart>), and any presence of melena or hematochezia was recorded by pen. Appetite was assessed by pen once daily approximately 4 hours after feeding. Any leftover food was recorded as a decreased appetite event. Complete physical and ophthalmic evaluations were repeated at study completion.

Clinical pathology evaluation

Blood was collected into EDTA and plain tubes in all dogs on days 0 and 29 for CBC, reticulocyte count, and biochemistry analysis. Complete blood count parameters and reticulocyte counts were determined by use of an automated hematologic analyzer (Advia 2120i; Siemens Corp). Biochemistry parameters were measured on an automated clinical analyzer (Vitros 4600; Ortho Clinical Diagnostics).

Plasma drug quantification

Blood samples were collected for determination of ketorolac and diclofenac total plasma concentrations at the following time points:

- Blood was collected for plasma drug quantification in all dogs at approximately 7 AM on day 0.
- On day 4, 2 dogs from each group were selected randomly for determination of peak plasma drug levels; blood was collected 5, 15, 30, 60, 90, and 120 minutes after the 4 PM administration of eye drops.
- For the remainder of the study (days 14, 21, and 28), blood sampling occurred at the time following the 4 PM drug administration at which the peak plasma drug level was observed on day 4 ($t = 15$ minutes; see Results section).

For all blood samples collected throughout the study, EDTA samples were centrifuged within 1 hour of collection at $1,237 \times g$ for 15 minutes at 4°C and supernatant plasma stored at -80°C until analysis.

Analytical standard solutions—Ketorolac and diclofenac analytical standards were purchased from Cayman Chemical. Ketorolac-d5 and diclofenac-d4 analytical internal standards were also purchased from Cayman Chemical. Stock solutions of ketorolac and diclofenac were prepared at a concentration of 1 mg/mL in dimethylsulfoxide using American Chemical Society-grade dimethylsulfoxide from Fisher Chemical. Working stock solutions of ketorolac and diclofenac were prepared at concentrations of 0.1, 0.5, 1, and 10 $\mu\text{g}/\text{mL}$ using Optima liquid chromatography-mass spectrometry (LC-MS)-grade acetonitrile from Fisher Scientific. Stock solutions of ketorolac-d5 and diclofenac-d4 were prepared at a concentration of 1 mg/mL using Optima LC-MS-grade acetonitrile from Fisher Scientific. A working stock solution mixture of ketorolac-d5 and diclofenac-d4 was prepared at concentrations of 1 $\mu\text{g}/\text{mL}$ of each compound using Optima LC-MS-grade acetonitrile from Fisher Scientific.

Calibration and quality control standards—Calibration standards containing both ketorolac and

diclofenac at equal concentrations were prepared at concentrations of 0, 2, 5, 8, 10, 20, 50, 75, and 100 ng/mL by spiking appropriate volumes of working stock solutions into blank control canine plasma (Beagle plasma, K2EDTA, gender pooled, $0.2 \mu\text{m}$ filtered; BioIVT). Quality control (QC) standards containing both ketorolac and diclofenac at equal concentrations were prepared at concentrations of 4, 25, and 80 ng/mL by spiking appropriate volumes of working stock solutions into blank control canine plasma. Ketorolac-d5 and diclofenac-d4 were added as the internal standards into each calibration and QC standard at a concentration of 20 ng/mL by spiking appropriate volumes of working stock solutions. The total volume of all spiking solutions added to each calibration and QC standard was $\leq 5\%$ of the total solution volume.

Plasma sample preparation—Plasma samples were prepared by protein precipitation. A 200- μL aliquot of plasma was added to a 1.5-mL Eppendorf-style polypropylene tube. Eight hundred microliters of Optima LC-MS-grade acetonitrile was added, and the tube was capped and manually vortex mixed for 30 seconds. The polypropylene tubes were next centrifuged at a relative centrifugal force of approximately $10,300 g$ for 5 minutes. A 750- μL aliquot of the resulting supernatant was transferred to a 16 X 100-mm glass test tube and concentrated to dryness with a stream of dry nitrogen gas at 35°C and 15 psi using a TurboVap LV Concentration Workstation (Biotage). The dry residue was reconstituted with 150 μL of 80/20 (v:v) water:methanol, both Optima LC-MS grade, from Fisher Scientific. The reconstituted extract was transferred to a high-performance liquid chromatography (HPLC) autosampler vial with a fixed glass insert. Five microliters of this prepared sample was injected for LC-MS analysis.

Liquid chromatography-mass spectrometry method—Liquid chromatography-mass spectrometry analysis was performed using a Vanquish Flex ultra HPLC (UHPLC) system interfaced with a TSQ Altis triple quadrupole mass spectrometer (Thermo Fisher Scientific).

The analytical column was a Thermo Fisher Scientific Accucore C18, 50 X 2.1 mm, 2.6 μm . Mobile phase A was water with 0.1% (v:v) formic acid, and mobile phase B was acetonitrile with 0.1% (v:v) formic acid. The water, acetonitrile, and formic acid were all Optima LC-MS grade from Thermo Fisher Scientific. The column was thermostated to 35°C , and the mobile phase flow rate was 0.4 mL/min. The UHPLC gradient started with a composition of 20% B, which was held for 0.5 minutes and then ramped linearly to 100% B at 3.5 minutes. The composition was held at 100% B for 0.5 minutes and then step decreased to 20% and held for 1 minute to re-equilibrate the column prior to the next injection.

Mass spectral data were acquired in positive ion electrospray mode with the following source settings: spray voltage = 3,900 V, sheath gas = 45 arbitrary units (Arb), auxiliary gas = 18 Arb, sweep gas = 1 Arb, ion transfer capillary temperature = 325°C ,

and vaporizer temperature = 350 °C. Both quadrupoles, Q1 and Q3, were operated at a nominal resolution of 0.7 full width at half maximum. The collision-induced dissociation gas pressure was 2 mTorr.

Mass spectral data were acquired in selected reaction monitoring mode. For ketorolac, the precursor ion was the ([M+H]⁺) ion at *m/z* 256.1 and the product ions were *m/z* 105.0 and 77.0. The collision energies for these transitions were 19 and 37 eV, respectively. Signal from both transitions was summed and integrated to yield peak areas used for quantitation. For diclofenac, the precursor ion was the ([M+H]⁺) ion at *m/z* 296.1 and the product ions were *m/z* 250.0 and 215.0. The collision energies for these transitions were 13 and 19 eV, respectively. Signal from both transitions was summed and integrated to yield peak areas used for quantitation. For the ketorolac-d5 internal standard, the precursor ion was the ([M+H]⁺) ion at *m/z* 261.1 and the product ions were *m/z* 110.0 and 82.0. The collision energies for these transitions were 19 and 37 eV, respectively. Signal from both transitions was summed and integrated to yield peak areas used for quantitation. For the diclofenac-d4 internal standard, the precursor ion was the ([M+H]⁺) ion at *m/z* 300.0 and the product ions were *m/z* 254.0 and 219.0. The collision energies for these transitions were 13 and 19 eV, respectively. Signal from both transitions was summed and integrated to yield peak areas used for quantitation. The dwell times for all selected reaction monitoring transitions was 60 milliseconds. The LC-MS method was validated with respect to intra- and interday accuracy and precision, lower limit of quantitation, recovery, and carryover. The lower limit of quantitation for both ketorolac and diclofenac was 2 ng/mL.

Reanalysis of samples with concentrations above the upper limit of quantitation—Study samples that initially gave results for ketorolac that were above the upper limit of quantitation were diluted and reanalyzed. These samples were diluted 1:5 with blank canine control plasma, reprepared, and reanalyzed.

Statistical analysis

Two-sample Wilcoxon rank sum tests were performed to determine differences in endoscopy scores, weights, fecal score, incidence of other GI adverse events, and clinicopathological variables post-treatment (compared to baseline) and between ketorolac- and diclofenac-treated dogs. Fecal scores, vomiting, decreased appetite events, and hematochezia or melena were all recorded and analyzed by pen (*n* = 3 for ketorolac and 3 for diclofenac) and not by individual dog. A nonparametric approach was chosen due to the small sample size. Furthermore, the individual effects of systemic concentrations of diclofenac and ketorolac were examined independently using linear regression techniques. All analyses were carried out using R version 3.5.1 (The R Foundation for Statistical Computing). *P* values < .05 were considered statistically significant.

Results

There were 3 neutered males and 2 spayed females in the diclofenac group and 3 neutered males and 3 spayed females in the ketorolac group. Baseline weights were not significantly different between treatment groups (diclofenac, 9.1 kg [range, 8.8 to 9.1 kg]; ketorolac, 7.8 kg [range, 7 to 11.1 kg]). Physical exam or ophthalmic exam abnormalities were not observed in any dog at baseline or at post-treatment assessment.

Clinicopathologic data

There were no clinically significant differences in CBC and biochemistry values between the 2 treatment groups at baseline (**Table 1; Supplementary Table S1**). Post-treatment Hct was significantly lower in the ketorolac group when compared to the diclofenac group, although only 1 dog had an Hct that dropped below the reference range (37%; *P* = .014). Post-treatment MCV was significantly lower in the ketorolac group when compared to the diclofenac

Table 1—Selected baseline (T0) and post-treatment (T29) clinicopathologic variables depicted as median (range).

	Ketorolac		Diclofenac		<i>P</i> value Post TX K vs D	<i>P</i> value K: pre vs post	<i>P</i> value D: pre vs post
	T0	T29	T0	T29			
Hct (RI, 37%–55%)	45.85 (40.6–47.7)	43.45 (36–47.6) ^a	45 (42–53)	48.55 (47.6–57.8) ^a	.014	.575	.111
Hemoglobin (RI, 12–18 g/dL)	15.55 (13.6–16)	15.05 (12.7–16.5)	15.7 (14.3–18.4)	16.3 (15.7–19.1)	.165	.937	.176
Absolute reticulocyte (X 10 ³ /μL)	43.4 (19.5–53.7)	38.8 (27.1–48.8)	32.2 (18.1–69.9)	26.55 (22.7–39.2)	.114	.485	.712
MCV (RI, 60–77 fL)	67 (64.3–67.9)	62.95 (59.8–69.6) ^a	68.5 (67.3–69.5) ^b	71.4 (69–74.3) ^{a,b}	.019	.078	.037
MCHC (RI, 32–36 gm/dL)	33.5 (33.3–34.4)	34.5 (33.3–36)	34.8 (34–35.3) ^b	33.05 (32.7–34.6) ^b	.087	.076	.032
Platelets (RI, 200 X 10 ³ –500 X 10 ³ /μL)	332 (276–407)	323 (276–450)	330 (264–399)	292.5 (245–321)	.257	.810	.190
Neutrophils (RI, 3 X 10 ³ –11.4 X 10 ³ /μL)	4.51 (4.21–9.31)	7 (3.83–10.32)	4.07 (3.13–6.26)	4.27 (3.39–4.51)	.067	.394	.905
BUN (RI, 10–30 mg/dL)	13.5 (11–16) ^a	16.5 (16–22) ^a	12 (11–15) ^b	17 (14–18) ^b	.850	.007	.015
Creatinine (RI, 0.5–1.5 mg/dL)	0.65 (0.6–0.8)	0.6 (0.6–0.8)	0.7 (0.6–0.8)	0.7 (0.7–0.8)	.094	.652	.343
BUN/creatinine	21.43 (15.71–23.33) ^a	26.67 (20–36.67) ^a	18.33 (15–21.43)	24.29 (20–25.71)	.058	.031	.125
Albumin (RI, 2.7–4.0 mg/dL)	3.3 (3.1–3.6)	3.15 (2.8–3.5)	3.4 (3.2–4)	3.4 (3.2–3.7)	.168	.227	.751

Significant differences between groups or time points are denoted by the same superscript letter. Bolded *P* values are statistically significant. RI = Reference interval. TX = Treatment.

group, again with only 1 dog having a post-treatment MCV below the reference range (59.8 fL; $P = .019$). Post-treatment BUN increased significantly from baseline in both ketorolac- and diclofenac-treated groups ($P = .007$ and $.015$, respectively) but remained within the reference range in all dogs. Post-treatment BUN/creatinine ratio increased significantly from baseline in the ketorolac-treated dogs ($P = .031$), with a post-treatment BUN/creatinine ratio exceeding 27 in 2 of the ketorolac-treated dogs but none of the diclofenac-treated dogs. Creatinine and albumin remained within the reference interval in all dogs, with no significant differences between treatment groups or time points.

Plasma drug levels

Ketorolac and diclofenac were undetectable in the plasma of all dogs at T0. In the 2 dogs per group for which day 4 peak plasma levels were determined, peak plasma levels of both diclofenac and ketorolac occurred approximately 15 minutes after topical ophthalmic administration and the drugs were detected in the plasma at all time points evaluated (**Supplementary Table S2**). Day 14, 21, and 28 peak plasma concentrations of diclofenac ($n = 5$) and ketorolac dogs (6) were as follows, expressed as median (range): diclofenac day 14, 21.1 ng/mL (8.75 to 29.9 ng/mL); day 21, 20.6 ng/mL (5.5 to 28.1 ng/mL); and day 28, 27.5 ng/mL (11.3 to 31.1 ng/mL); and ketorolac day 14, 191 ng/mL (156 to 261 ng/mL); day 21, 173.5 ng/mL (142 to 233 ng/mL); and day 28, 179.5 ng/mL (135 to 210 ng/mL). At some time points, diclofenac and ketorolac were both detectable and quantifiable in a single dog (**Figure 1**; **Supplementary Table S3**).

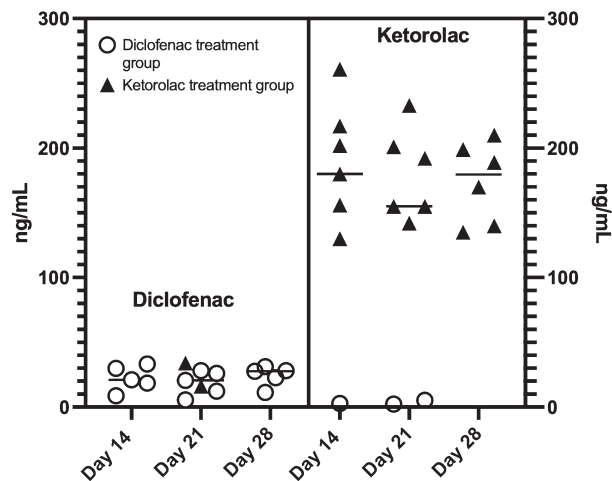


Figure 1—Plasma diclofenac (left side) and ketorolac concentrations (right side) expressed in ng/mL. Horizontal lines represent median concentrations.

Regardless of treatment group assignment, plasma ketorolac levels were significantly related to the total ($P = .015$) and antral endoscopy scores ($P < .001$). There was no significant relationship between plasma diclofenac levels and endoscopy score at any site (**Figure 2**).

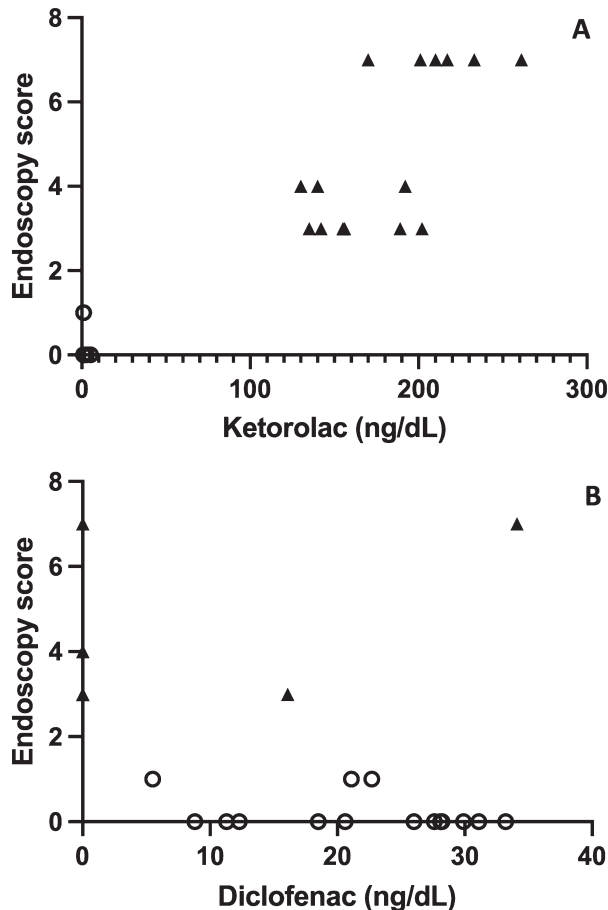


Figure 2—Relationship between plasma ketorolac concentrations (A) and plasma diclofenac concentrations (B) measured throughout the study duration and antral endoscopy score documented post-treatment (T29). Triangles represent data points from dogs assigned to the ketorolac treatment group, and open circles represent data points from dogs assigned to the diclofenac treatment group.

Gastroduodenoscopy

There were no significant differences in endoscopy scores between ketorolac- and diclofenac-treated dogs at baseline (**Table 2**). No dogs had ulcers at baseline. One dog in each treatment group had erosions (stomach body and fundus) at baseline, one of which was possibly associated with an incidental gastric foreign body in a dog assigned to the ketorolac treatment group that was removed at the time of baseline endoscopy.

Post-treatment, ulcers and/or erosions developed in 1 of 5 diclofenac-treated dogs and 6 of 6 ketorolac-treated dogs (**Figure 3**). The post-treatment total endoscopy score and post-treatment antral score were significantly higher ($P = .042$) in the ketorolac-treated dogs when compared to baseline (**Figure 4**). Post-treatment endoscopy scores in diclofenac-treated dogs did not differ significantly from baseline. Post-treatment endoscopy scores were significantly higher ($P = .007$) in the antrum in ketorolac-treated dogs than in diclofenac-treated dogs but were not significantly different at any other site (**Table 2**; **Figure 4**).

Table 2—Median (range) T0 and T29 endoscopic mucosal scores.

Site	Ketorolac		Diclofenac		P value
	T0	T29	T0	T29	
Esophagus	0	0	0	0	—
Stomach body	2 (0-3)	2.5 (1-6)	2 (1-2)	2 (0-7)	.842
Antrum/body junction	0 (0-2)	0 (0-2)	1 (0-1)	0 (0-2)	.521
Antrum	0 (0-1) ^a	4 (3-7) ^{a,b}	0 (0-2)	0 (0-1) ^b	.006
Retroflex	1 (0-2)	0.5 (0-3)	1 (1-3)	0 (0-2)	.763
Duodenum	0	0 (0-7)	0	0	.361
Total	4 (1-6) ^b	7.5 (4-20) ^b	4 (3-7)	2 (0-9)	.042

Significant differences between groups or time points are indicated by the same superscript letter. Bolded *P* values are statistically significant.

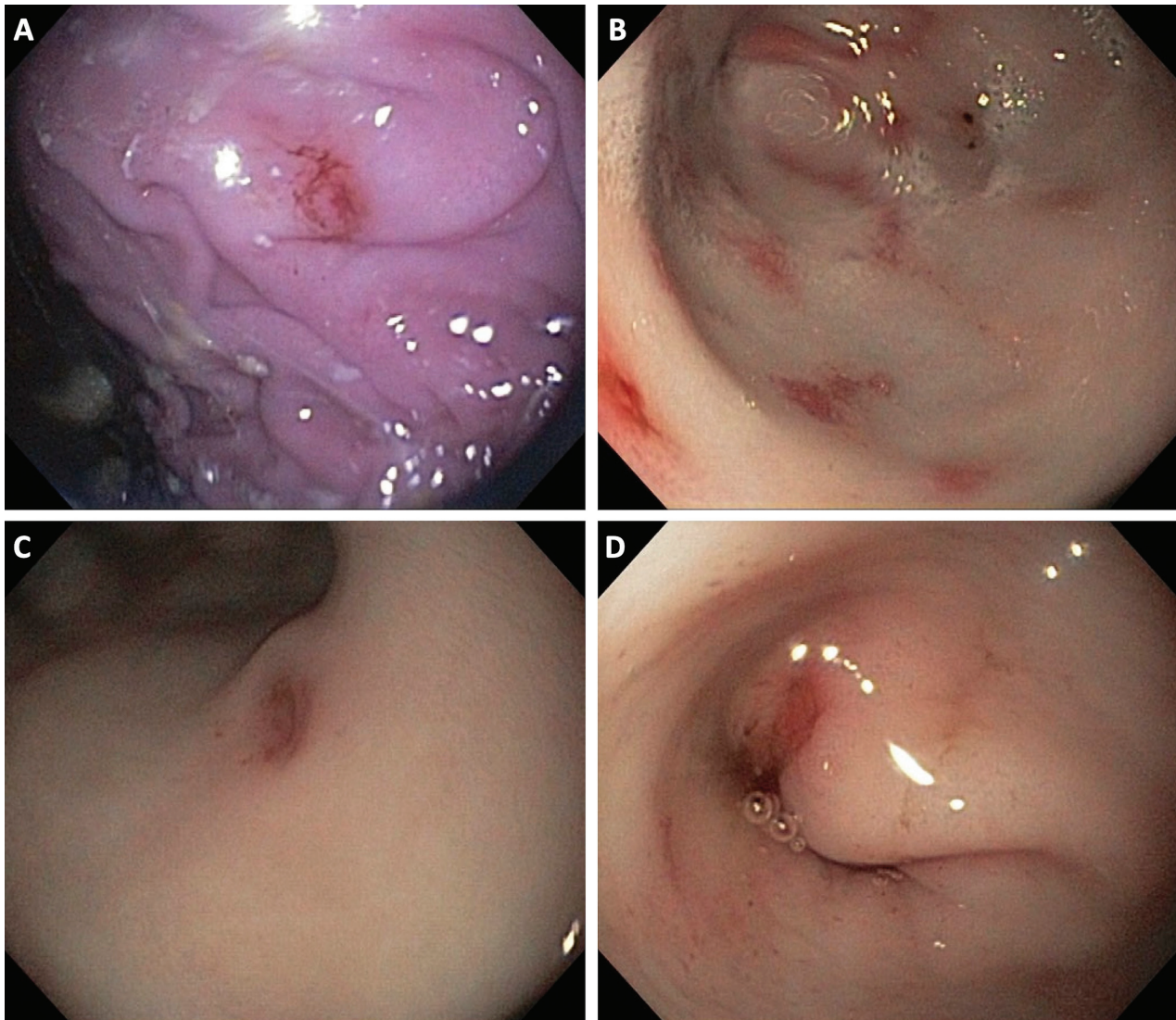


Figure 3—Representative mucosal lesions documented during post-treatment endoscopy. A—Gastric body ulcer in a diclofenac-treated dog. B—Antral erosions in a ketorolac-treated dog. C and D—Antral ulcers in ketorolac-treated dogs.

Other GI adverse effects

Guaiac fecal occult blood test (FOBT) was negative in all dogs at all time points. Vomiting, decreased appetite events, and melena or hematochezia were uncommonly observed during the study and were not significantly different between treatment groups. There was a total of 3 observed vomiting events, 3

decreased appetite events, and 1 hematochezia event. There was no observed melena or hematemesis. All observed GI adverse effects occurred in ketorolac-treated dogs. Post-treatment weight did not differ significantly from baseline, and there was no difference in the change in body weight between ketorolac- and diclofenac-treated dogs.

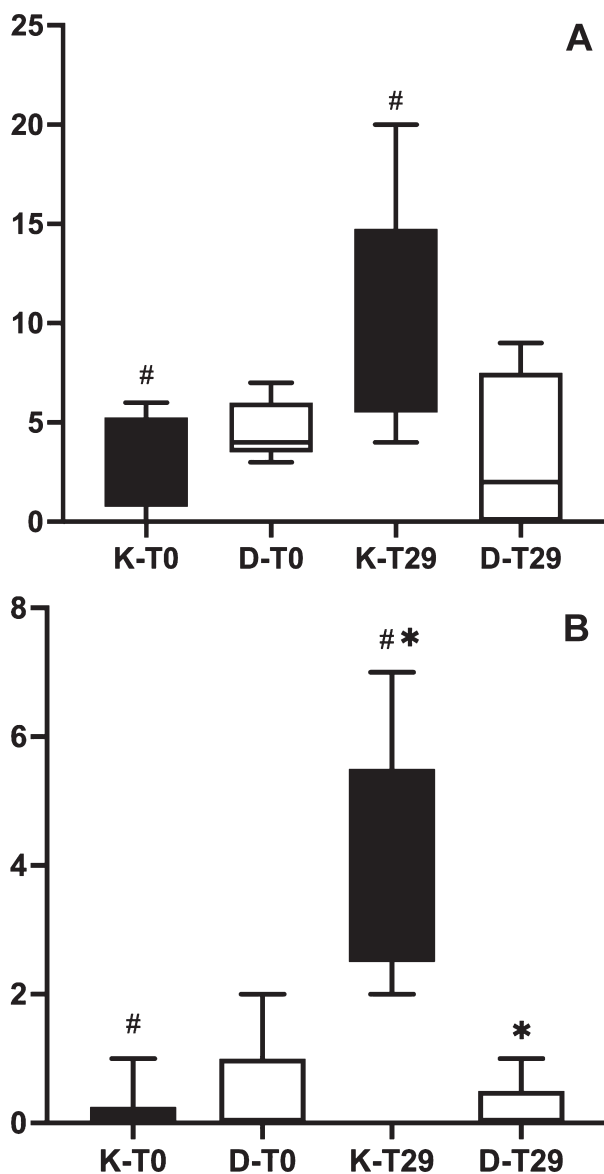


Figure 4—Box-and-whisker plots depicting baseline (T0) and T29 total endoscopy scores (A) and antral endoscopy scores (B) of diclofenac- and ketorolac-treated dogs. Statistically significant differences are denoted by the pound sign and an asterisk. Whiskers depict the maximum and minimum, boxes depict the IQR, and the horizontal line represents the median.

Discussion

Ophthalmic NSAIDs are often administered to dogs with the assumption that systemic absorption, and therefore the risk of systemic adverse effects, is negligible. In this study, we found that the topical ophthalmic administration of ketorolac resulted in consistently high plasma levels and associated GI mucosal lesions in healthy dogs.

Ketorolac is a potent nonselective cyclooxygenase inhibitor that, when given systemically, is intended as an alternative to narcotic analgesics for the treatment of moderate to severe pain. Because of its

high risk of GI ulceration in humans relative to other NSAIDs, it is recommended to limit administration to no more than 5 days.^{11,12} It is reasonable to suspect that ketorolac, when absorbed in high enough levels, is likely to be more ulcerogenic than many other NSAIDs in dogs as well as in humans.

Throughout this study, ketorolac attained much higher plasma concentrations than diclofenac. The high plasma levels attained by ketorolac in this study were important since the systemic concentration of drug likely drove the GI lesions. We found a strong relationship between the plasma ketorolac concentration and the magnitude of GI endoscopic lesion score, but no such relationship was found for diclofenac (Figure 2). The high plasma concentrations of ketorolac observed in this study can be partly explained by the higher dosage of the drug given to the ketorolac-treated group, due to the difference in concentration between the 2 products (0.5% ketorolac tromethamine vs 0.1% diclofenac sodium). However, the plasma concentrations of ketorolac were often found to be 10 times higher than those of diclofenac, which cannot be solely attributed to the 5-fold difference in drug concentration (Figure 1). The reason for the higher plasma concentration of ketorolac is unknown, but it is likely due (at least in part) to differences in the elimination half-life between the 2 NSAIDs. Indeed, the elimination half-life of ketorolac is reportedly longer than for diclofenac in canine subjects, although the pharmacokinetics data are sparse and involve different cohorts of dogs and routes of administration.^{14,15,22,23} With repeated drug administration (ie, 4 times daily eye drops for weeks), a longer half-life of ketorolac would translate into higher plasma levels due to drug accumulation in the body. Note that differences in the elimination half-life between these drugs could be attributed to either a faster systemic clearance of diclofenac, a larger volume of distribution of ketorolac, or a combination of the two.

Interestingly, the peak plasma concentrations of ketorolac reported in our dogs overlapped with those found in studies documenting effective analgesia after IV or PO dosing of ketorolac in this species.^{14,15} In contrast, peak plasma levels of diclofenac were far lower than those reported after PO or IM dosing in dogs.^{22,23} Pharmacokinetic and pharmacodynamic data on systemically administered diclofenac in dogs are limited at the time of this writing, and it is not known at what plasma concentrations systemic effects (favorable or adverse) would be expected to occur. Interestingly, the plasma concentrations of diclofenac in our dogs were also far lower than those reported after ophthalmic administration in cats.¹⁷ This was due in part to the higher daily mg/kg dose of diclofenac administered to the cats due to their smaller size compared to our dogs and potentially to species differences in pharmacokinetics between dogs and cats (ie, due to differences in drug bioavailability, systemic clearance, and/or volume of distribution). In another study²⁴ of rabbits, diclofenac and ketorolac were administered for 90 days at a daily mg/kg dose similar to that in our study; while

systemic absorption was only determined qualitatively in that study, none of the rabbits developed GI lesions or adverse effects with either NSAID. As such, it is important for clinicians and scientists to examine potential systemic adverse effects of topical NSAIDs (or other ophthalmic drugs) in the species of interest, as direct extrapolation between species is biased by key species differences in ocular anatomy and physiology.²⁵

Despite the finding of ulcers and erosions in many dogs, fecal occult blood was not detected in the feces of any dog at any time point throughout the study. This was relatively unsurprising. In a past study²⁰ demonstrating the development of endoscopic lesions associated with NSAID administration in healthy dogs, FOBT was similarly negative throughout the study, which was attributed to too little blood in the feces to be detected by the test. The package insert reports that the test used in this study was expected to be positive, with daily blood loss at or exceeding 10 mL in healthy adult humans.¹⁹ In a recent study²⁶ of dogs, the rate of positivity was associated with the dose of blood, with most, but not all, seen at the highest dose of 40 mg of hemoglobin/kg of body weight. In this study, given the small lesions, it is reasonable to suspect that the test was not sensitive enough to detect the small amount of blood lost from the lesions.

Other GI adverse effects such as vomiting and decreased appetite were uncommon in this study, and there were no statistical differences between treatment groups. This is unsurprising given how common it is for GI mucosal lesions in association with NSAIDs to occur in the absence of overt clinical signs.^{27,28}

Overall, CBC and biochemistry changes in this study were uncommon. Since GI ulceration and erosion secondary to systemic absorption of NSAIDs was the primary point of interest in this study, it is noteworthy that the strongest statistically significant findings included a post-treatment increase in BUN and increase in BUN/creatinine ratio, including an increase above a cutoff that has been reported in association with upper GI bleeding in dogs and in humans.²⁹ While the most straightforward explanation for these findings is increased protein absorption due to NSAID-induced GI bleeding, this is questionable for multiple reasons. First, only 1 observation of gross blood was noted during the study and that was hematochezia. Second, none of the FOBT were positive at any time point. Furthermore, a recent study³⁰ demonstrated that BUN/creatinine ratio did not increase in dogs with occult GI bleeding. It is possible that subtle, subclinical changes in volume status associated with vomiting and theoretical decreased water intake (not quantified) because of dyspepsia from the NSAIDs resulted in a preferential increase in BUN due to volume contraction and not GI bleeding at all. Alternatively, it is possible that there were more extensive slow-bleeding lesions distal to those observed on upper GI endoscopy that contributed to the increase in BUN and BUN/creatinine ratio post-treatment, although the FOBT results do not support this theory.

The data presented here must be interpreted in the context of several important limitations. First,

this study did not include a placebo control group. It is possible that other factors unrelated to systemic absorption of ophthalmic NSAIDs contributed to the development of ulcers and erosions in these dogs. In previous studies evaluating the GI mucosa in healthy dogs administered NSAIDs, some lesions were found at baseline or after administration of placebo, indicating the stress of the study procedures themselves or other factors unrelated to NSAIDs were contributing to the development of these lesions.^{20,27} While our dogs were already acclimated to their diet and housing environment, a specific acclimation period to this study design prior to implementation of data collection (ie, 4 times daily administration of saline drops), was not done. It is possible that this could have contributed to GI mucosal lesions independent of systemic absorption of NSAIDs, although this would not explain the difference in lesion number and severity seen in the ketorolac-treated group.

Additionally, some dogs had time points when significant plasma levels of both their assigned NSAID and the NSAID of the opposite treatment group were detected (Figure 1; Supplementary Table S2). This is problematic because simultaneous administration of 2 different NSAIDs is generally not recommended and may be associated with a greater risk of adverse effects.³¹ Finding low levels of ketorolac in the plasma of dogs in the diclofenac treatment group was common, but usually well below the plasma levels attained by the ketorolac dogs, and often so low that it was not quantifiable. Reasons for this are unclear but suspected to be due to environmental contamination. While dogs were only pair-housed with dogs assigned to the same treatment, the pens were adjacent and it is possible that low levels of ketorolac could have been picked up in the environment. While efforts were made to keep treatment groups separate at all times, it is possible that dogs let out during cleaning procedures could have come into contact with feces of dogs from the opposite treatment group. In contrast, detectable diclofenac in the plasma of ketorolac-treated dogs was noted in 2 dogs on day 21. In these dogs, diclofenac was detected at plasma levels comparable to the diclofenac-treated dogs, suggesting that these dogs were inadvertently administered the incorrect eye drops at that time point. These 2 dogs had concurrent plasma levels of ketorolac at a magnitude consistent with those seen in the ketorolac-treated dogs. Regardless of the treatment assigned, plasma ketorolac concentrations were strongly related to severity of GI mucosal lesions while diclofenac concentrations were not, suggesting the GI lesions resulted from high plasma ketorolac levels and not exposure to multiple NSAIDs.

Another study limitation was the assessment of dogs with healthy eyes, which may have underestimated the systemic absorption and potential adverse effects. Indeed, canine eyes with various pathologies requiring topical NSAIDs (eg, conjunctivitis, uveitis, cataracts) are known to have "leaky" conjunctiva, that is, a breakdown of the blood-tear barrier that might allow tear film compounds to

reach the systemic circulation at higher levels and vice versa.^{25,32–35} Finally, the small sample size must be taken into consideration when interpreting the statistical relationship found between endoscopic scores and plasma ketorolac concentrations.

In conclusion, this study provided evidence that topical administration of ketorolac ophthalmic solution results in high plasma concentrations and associated GI mucosal lesions. Dogs administered ophthalmic ketorolac should be monitored for GI adverse effects, and ketorolac should be used with caution in dogs with risk factors for GI ulceration and bleeding. Further studies are needed to determine safety guidelines for clinical populations as well as strategies to reduce systemic absorption of this drug while maximizing anti-inflammatory benefits to the eye.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.