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The Effect of Tramadol and Indomethacin Coadministration on Gastric Barrier Function in Dogs

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Background: Tramadol is a centrally acting analgesic that is often used in conjunction with nonsteroidal anti-inflammatory drugs (NSAIDs). The effect of coadministration of tramadol and indomethacin on gastric barrier function in dogs is unknown.

Hypothesis/Objectives: That coadministration of a nonselective NSAID (indomethacin) and tramadol would decrease recovery of barrier function as compared with acid-injured, indomethacin-treated, and tramadol-treated mucosa.

Animals: Gastric mucosa of 10 humanely euthanized shelter dogs.

Methods: Ex vivo study. Mounted gastric mucosa was treated with indomethacin, tramadol, or both. Gastric barrier function, prostanoid production, and cyclooxygenase expression were quantified.

Results: Indomethacin decreased recovery of transepithelial electrical resistance after injury, although neither tramadol nor the coadministration of the two had an additional effect. Indomethacin inhibited production of gastroprotective prostanoids prostaglandin E_2 (acid-injured PGE_2: 509.3 ± 158.3 pg/mL, indomethacin + acid injury PGE_2: 182.9 ± 93.8 pg/mL, $P < .001$) and thromboxane B_2 (acid-injured TXB_2: 233.2 ± 90.7 pg/mL, indomethacin + acid injury TXB_2: 37.9 ± 16.8 pg/mL, $P < .001$), whereas tramadol had no significant effect (PGE_2 $P = .713$, TXB_2 $P = .194$). Neither drug had an effect on cyclooxygenase expression (COX-1 $P = .743$, COX-2 $P = .705$). Acid injury induced moderate to marked epithelial cell sloughing, which was unchanged by drug administration.

Conclusions and Clinical Importance: There was no apparent interaction of tramadol and a nonselective cyclooxygenase in this ex vivo model. These results suggest that if there is an adverse interaction of the 2 drugs in vivo, it is unlikely to be via prostanoid inhibition.

Key words: NSAID; tramadol; ulcer.

As recognition of acute and chronic pain in dogs has increased, so too has the desire to optimize pain treatment. This is frequently achieved by use of nonsteroidal anti-inflammatory drugs (NSAIDs), but in some cases, multimodal treatment is attempted using centrally acting analgesics, such as tramadol. NSAIDs are a common cause of gastroduodenal ulceration in people and dogs. In dogs, mortality associated with NSAID-induced gastroduodenal perforation is up to 70% in 1 study, but the overall incidence of ulceration with NSAID treatment is unknown.[1] Ulceration induced by NSAIDs is primarily because of inhibition of gastroprotective prostanoids elaborated by the COX enzymes. Gastroduodenal ulcers have been observed in dogs treated with both nonselective and selective COX-2 inhibitors.[2]

It has been suggested that tramadol treatment increases the risk of peptic ulcer perforation in people.[3] There are anecdotal reports that dogs concurrently receiving an NSAID and tramadol have a higher prevalence of gastric and duodenal perforations as compared with dogs treated with NSAIDs alone.[4]

Abbreviations:

COX	cyclooxygenase
ELISA	enzyme-linked immunosorbent assay
Isc	short circuit current
NSAID	nonsteroidal anti-inflammatory drug
PD	potential difference
PGE_2	prostaglandin E_2
TER	transepithelial electrical resistance
TXB_2	thromboxane B_2

In rats, the combination of rofecoxib, a COX-2 selective inhibitor, and tramadol produced at least twice as many gastric ulcers than either drug administered separately.[5] The mechanisms for this interaction are unknown, although they might be related to tramadol's effects on opiate receptors in the gut.[5] Tramadol has many metabolites in the dog.[6] The possible gastrointestinal adverse effects of tramadol and its metabolites have not been described.

Mucosal barrier function can be assessed by measurement of transepithelial electrical resistance (TER) in Ussing chambers. Additionally, barrier function can be assessed using flux of a larger molecule, such as mannitol or dextrans, which is radiolabeled or fluorescently tagged and can only move paracellularly. In dogs, this model system has been previously used to examine effects of carprofen and meloxicam.[7] Using an ex vivo model of acid-induced gastric barrier dysfunction, we sought to investigate the effect of the parent compound tramadol on gastric barrier function as well as its potential interaction with a nonselective COX inhibitor, indomethacin. We hypothesized that tramadol would have an additive or synergistic effect with

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a nonselective COX inhibitor, indomethacin, in decreasing recovery of barrier function, as assessed by transepithelial resistance and ^{3}H-mannitol flux, after injury.

Materials and Methods

Tissue Collection

Tissue samples were obtained from a convenience sample of 10 dogs previously scheduled for euthanasia by shelter veterinarians. Dogs were included if they are approximately 10 months to 7 years of age, 10–35 kg, and appeared normal on physical examination. Dogs were euthanized according to AVMA-approved guidelines selected by shelter veterinarians. Immediately after euthanasia, a midline celiotomy was performed and the stomach was exteriorized. The gastric antrum was excised along the greater curvature from the pyloric sphincter to the incisura angularis and placed mucosal side down into oxygenated (95% O_2, 5% CO_2) Ringer's solution at room temperature. Approximately 20–30 minutes later, the tissue was transferred to dissection pans in the laboratory and bathed in fresh oxygenated Ringer's solution.

Ussing Chamber

The antral mucosa was dissected from the seromuscular layer and mounted in Ussing chambers (1.14 cm^2 surface area). One mucosal sample was used from each dog for each treatment (Ussing chamber conditions). Canine Ringer's solution contained (in mM): 112.0 NaCl, 4.0 KCl, 2.4 $CaCl_2$, 0.8 $MgCl_2$, 25.0 $NaHCO_3$, 0.23 NaH_2PO_4, and 1.58 Na_2HPO_4. Ten mmol/L glucose was added to the serosal bathing solution to maintain viability of the tissue and balanced with 10 mmol/L mannitol in the mucosal bathing solution. Tissue was maintained at 37°C in chambers bathed with oxygenated Ringer's in water-jacketed reservoirs. After a 30-minute equilibration period, tissue was injured by application of Ringer's solution titrated to a pH of 1.2 with HCl to the mucosal side of the tissue for 45 minutes.

Transepithelial Resistance

The spontaneous potential difference (PD) was measured with Ringer-agar bridges connected to calomel electrodes, and the PD was short-circuited through silver-silver chloride electrodes with a voltage clamp that corrected for fluid resistance to calculate short-circuit current (Isc). If the spontaneous PD was between −1 mV and 1 mV, tissues were current-clamped at ± 100 μA for 5 seconds and the PD was recorded. The Isc and PD were recorded every 15 minutes for 210 minutes. Data were entered into spreadsheets that calculated TER from Isc and PD using Ohm's law. One chamber was maintained with neutral pH Ringer's solution as a control.

Ussing Chamber Treatments

After 45 minutes of acid injury, acidified Ringer's was replaced with neutral Ringer's solution. Immediately after acid injury, drug treatments were applied. Drug treatments were one of the following: indomethacin 10^{-5} M, tramadol 10^{-6} M, and indomethacin + tramadol at 10^{-5} and 10^{-6} M, respectively. The selected doses for indomethacin and tramadol are 2- to 10-fold higher than reported maximum serum concentrations for these drugs.[8,9] Drugs were applied to mucosal and serosal bathing reservoirs to mimic both topical and systemic effects of each drug. Controls included uninjured tissue with no drug treatment and acid-injured tissue without drug treatment.

^{3}H-Mannitol Flux

As a second indicator of gastric permeability, flux of ^{3}H-labeled mannitol across the mucosa was measured. A total volume of 200 uM of ^{3}H-radiolabeled mannitol was added to the mucosal reservoir. Samples were taken of both serosal and mucosal reservoirs after 3 minutes to establish baseline radioactivity. Two 1-hour mucosal to serosal fluxes were performed by sampling serosal bathing solutions at 1 and 2 hours after addition of radiolabeled mannitol.

Prostanoid Levels

Samples of the serosal bathing solutions were collected at 30 and 210 minutes of tissue incubation after which they were snap-frozen in liquid nitrogen and stored at −80°C until analysis. The amounts of thromboxane B_2 (TXB_2, the stable metabolite of TXA_2) and prostaglandin E_2 (PGE_2) were measured using ELISA.[a]

Western Blot

Western blot analyses for COX-1 and -2 were completed using gastric mucosal tissue obtained at 0 and after 210 minutes and semiquantization using densitometry. β-actin expression was used as a loading control to determine relative quantification. Sheep COX-1 and recombinant human COX-2 were used as positive controls. After transfer and blocking with 5% milk for 2 hours, goat COX-1 and COX-2 antibodies[b] were applied at 1 : 150 in a 5% milk solution and incubated overnight at 4°C. Donkey anti-goat secondary antibodies at 1 : 3,000 in 5% milk were then applied for 1 hour and developed.

Histologic Examination

Gastric mucosal samples were taken for each dog before mounting tissue on Ussing chambers for baseline histologic evaluation. After 210 minutes, the tissues were collected from each of the 5 treatment groups and placed in 10% neutral buffered formalin. All 6 samples (baseline plus 5 treatment groups) from each dog were routinely processed, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and viewed with a light microscope by a pathologist (J.M.L.).

Data Analysis/Interpretation

A 2-way ANOVA was used to compare transepithelial resistance data among the 5 treatment groups (control, acid Ringer's, tramadol, indomethacin, and tramadol + indomethacin) over the time period the tissues were in the Ussing chambers using Tukey's test for posthoc analysis. A Kruskal-Wallis 1-way ANOVA on ranks was used to analyze western blot results and prostanoid concentrations. The Tukey posthoc test was used to detect differences among treatments at different times. Results are expressed as mean ± SD. Significance was set at $P < .05$.

Results

Acid injury induced a significant and partially reversible decrease in barrier function as assessed by TER (Fig 1). At point of maximal acid-induced change in barrier function, TER of mucosa treated

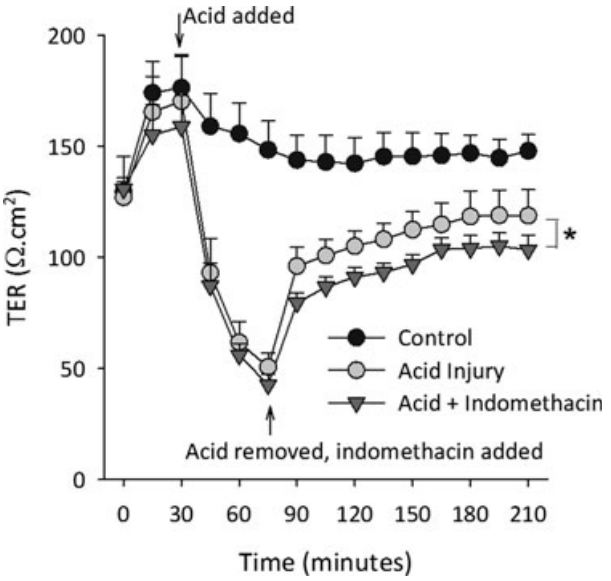

Fig 1. Acid injury induces a significant decrease in TER followed by partial recovery (90–210 minutes). Indomethacin decreased recovery of TER after injury as compared with acid-injured control ($P = .034$). N = 10, values represent mean ± SE.

with acid injury was 34.9 ± 5.3% of control. At 210 minutes, TER of acid-injured tissue was 83.9 ± 9.7% of control tissue. There was an overall significant effect of treatment on TER recovery after injury ($P < .001$). Acid-injured tissue treated with indomethacin after injury recovered significantly less than acid-injured control (Fig 1, $P = .034$). Tramadol, with or without concurrent indomethacin administration, did not significantly affect TER recovery after injury (Figs 2 and 3).

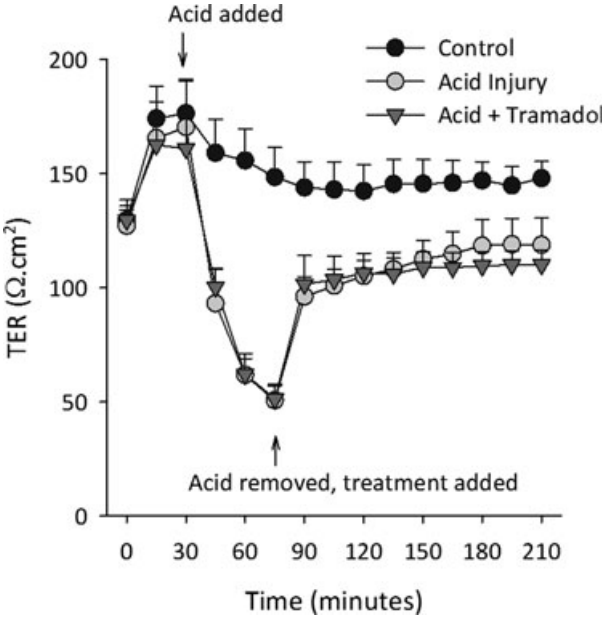

Fig 2. Tramadol had no significant effect on recovery of TER after acid injury. N = 10, values represent mean ± SE.

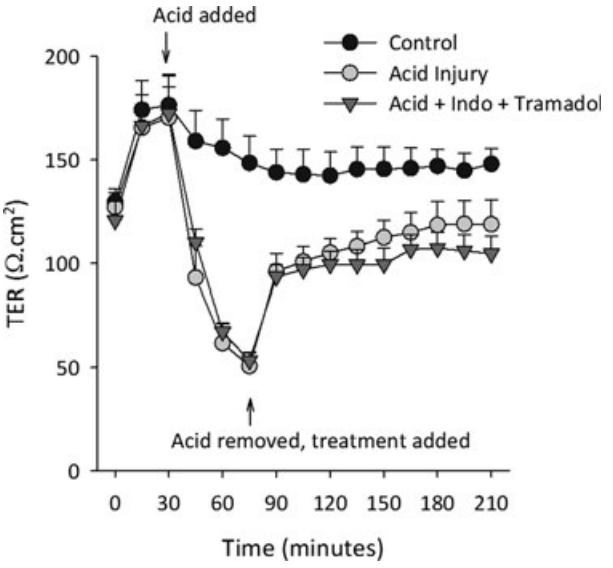

Fig 3. Indomethacin and tramadol coadministration had no significant effect on recovery of TER after acid injury. N = 10, values represent mean ± SE.

Flux of ^{3}H-mannitol after acid injury, with or without drug administration, was not significantly different than control (data not shown).

There was a significant effect of treatment on the change in concentrations of both PGE_2 and TXB_2 (PGE_2: $P < .001$, TXB_2: $P < .001$). Acid injury increased synthesis of PGE_2 (control PGE_2: 65.7 ± 26.8 pg/mL, acid-injured PGE_2: 509.3 ± 158.3 pg/mL,

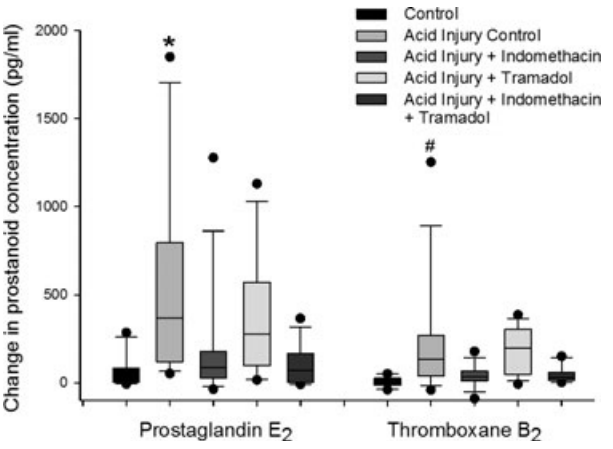

Fig 4. Change in prostanoid concentration from baseline (30 minutes) to 210 minutes is pictured. There was a significant effect of treatment on both PGE_2 and TXB_2 concentrations ($P < .001$). Acid injury induced an increase in PGE_2 and TXB_2. Indomethacin attenuated this increase when administered alone or concurrently with tramadol. Tramadol had no effect on PGE_2 or TXB_2 concentrations induced by acid injury when administered alone or concurrently with indomethacin. *PGE_2 of acid injury control was higher than uninjured control, indomethacin, and indomethacin/tramadol in pairwise comparisons ($P < .05$). $^{\#}TXB_2$ of acid-injured control was greater than uninjured control in pairwise comparisons ($P < .05$). N = 10, values represent mean ± SE.

Fig 4); this increase was attenuated when acid-injured tissue was treated with indomethacin or indomethacin + tramadol (indomethacin + acid injury PGE_2: 182.9 ± 93.8 pg/mL, indomethacin + tramadol + acid injury PGE_2: 99.7 ± 31.6 pg/mL). There was no significant effect of tramadol on PGE_2 concentration (384.1 ± 95.8 pg/mL).

TXB_2 also increased with acid injury (control TXB_2: 7.1 ± 7.8 pg/mL, acid-injured TXB_2: 233.2 ± 90.7 pg/mL, Fig 4). Similar to PGE_2, indomethacin treatment with acid injury, with or without tramadol coadministration produced thromboxane levels similar to uninjured control (indomethacin + acid injury TXB_2: 37.9 ± 16.8 pg/mL, indomethacin + tramadol + acid injury TXB_2: 47.5 ± 12.7 pg/mL). There was no effect of tramadol on TXB_2 concentration (188.5 ± 35.5 pg/mL).

COX-1 and COX-2 were both present at baseline in canine gastric mucosa (Fig 5). There was no effect of the 210-minute ex vivo experiment on either COX-1 or -2 protein expression. Acid injury, with or without drug administration, did not change expression of either COX-1 or COX-2 enzyme.

Acid injury induced diffuse moderate to marked sloughing of luminal gastric epithelial cells (Fig 6). There was no apparent additional effect of either indomethacin or tramadol on tissue morphology with acid injury.

Discussion

In this ex vivo model, there was no effect of tramadol on recovery of gastric barrier function, as assessed by TER. Additionally, there was no apparent interaction between indomethacin and tramadol, although indomethacin alone slightly decreased recovery of barrier function as measured by electrical resistance (TER). TER measures the movement of ions across an epithelial membrane. With mucosal injury, the tight junctions that normally would provide resistance to the movement of ions might be altered, decreasing barrier function. It was unexpected that indomethacin did not have a stronger effect on barrier function, given that it decreased the production of prostanoids so dramatically. Gastric mucosa has multiple non-COX–dependent recovery mechanisms, including increased mucus production and alterations in tight junction morphology, which could explain a lower detrimental effect of indomethacin than might be expected. It is also possible that canine mucosa is more resistant to adverse effects of COX inhibition than mucosa of other species previously examined in this system (pig, mouse, horse). Additionally, previous work using indomethacin has largely been focused on effects on intestinal epithelium and gastric epithelium might be more resistant to COX inhibition in this model.

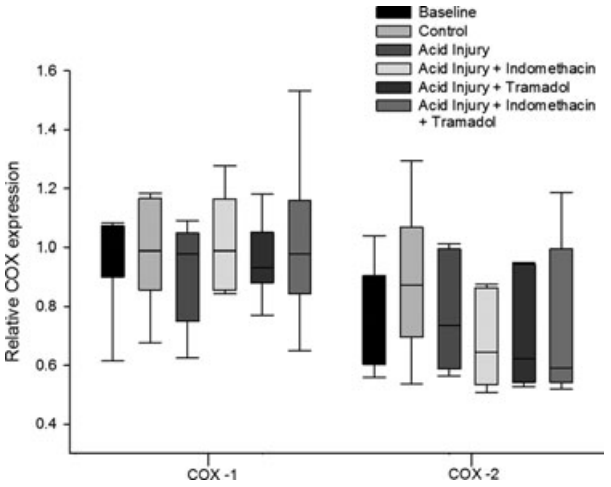

Fig 5. There was no change in COX-1 and -2 expression from baseline to the end of the experimental period (baseline versus control). There was also no significant effect of any treatment (acid injury, indomethacin, tramadol) on COX-1 or -2 expression. Both isozymes were expressed at baseline (before injury or mounting on the chambers).

Likewise, tramadol had no significant effect on recovery after acid injury. Although tramadol's effect on barrier function has not been previously examined, there is a suggestion in rat models and humans that tramadol might predispose to gastroduodenal ulceration. In the current study, only the parent tramadol compound was applied to the tissue. There are over 20 metabolites of tramadol with unknown activity in dogs.[10] It is possible that while the parent compound alone does not affect TER or prostanoid synthesis, one of its metabolites might. There has, to date, been no reported information regarding the effect of metabolites on mucosal barrier function. The metabolites were not examined because of lack of commercial availability or cost.

Doses selected for both indomethacin and tramadol were determined using data from preliminary studies. Dose-response studies in 5 dogs before this work did not show a significant effect of treatment at this and 2 lower doses (10^{-6} and 10^{-7} M for indomethacin, 10^{-7} and 10^{-8} M for tramadol, data not shown). In our preliminary work, the highest dose of indomethacin with tramadol tended to decrease TER recovery; the highest concentration of both drugs was therefore selected for the study. Higher doses than reported maximal serum concentrations were used in this study to give the greatest likelihood of appreciating an ex vivo interaction. Although these doses are higher than maximal reported serum concentrations for either drug, it is uncertain how this model might alter drug effects ex vivo. It might be that higher concentrations than those used in this study would have shown a greater effect on TER.

No treatment had an effect on the second measure of gastric barrier function, flux of ^{3}H-mannitol. This molecule is larger, so is relatively less sensitive to small changes in barrier function as compared with TER. The lack of significant effect may signify the role of non-COX dependent mechanisms in gastroprotection and the relative strength of the gastric mucosa to protect against acid-mediated injury.

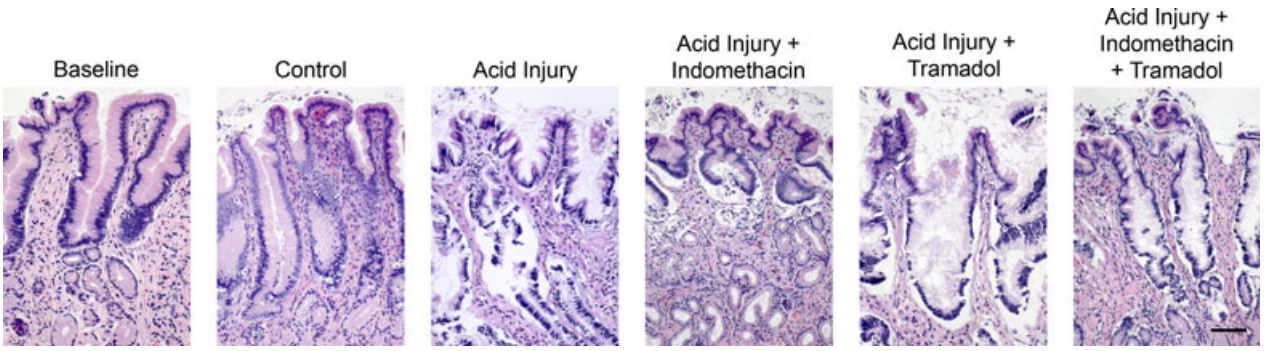

Fig 6. Acid injury induced moderate to marked superficial gastric epithelial sloughing. There was no apparent effect of any treatment on tissue morphology. Hematoxylin and eosin stain. Bar = 100 μm.

Indomethacin was selected for this model because of its properties as a nonselective COX inhibitor. Although not clinically utilized, indomethacin is a commonly used COX inhibitor in ex vivo and in vitro assays. Additionally, it was felt that a nonselective inhibitor would be more likely to show an interaction with tramadol than a more selective inhibitor. However, it is a limitation of the study that we were unable to examine the effect of a COX-2 selective inhibitor along with a nonselective COX inhibitor. It may be that a COX-2 selective inhibitor may have shown different effects.

COX expression itself was unchanged by any treatment, including mounting of the tissue on Ussing chambers for 210 minutes, although this is an index of COX expression, not an assay of COX activity. It might be that the activity of one or the other might have been altered by treatment, but this was not quantified in the current study. Additionally, 210 minutes might not be enough time to appreciate a significant change in COX protein expression. Interestingly, both COX-1 and COX-2 were present at baseline and expression was not significantly different between the 2 isozymes. The paradigm of COX-1 as "constitutive" and COX-2 as "inducible" seems to be fading as evidence mounts that there is an overlap between the two and both are important in times of health and injury.[11,12]

There is no evidence, using this canine ex vivo model of acid injury, that tramadol alone has a detrimental effect to gastric barrier function. Concentrations of both prostanoids measured, PGE_2 and TXB_2, were unchanged in acid-injured, tramadol-treated tissue versus acid injury control. Similarly, COX-1 and -2 expression was unaffected by tramadol administration. Additionally, the parent compound tramadol apparently did not induce a tissue reaction that increased production of gastroprotective prostanoids, although metabolites of tramadol were not assessed. The parent compound, tramadol, does not adversely affect gastric barrier function ex vivo in the dog, either alone or in conjunction with a nonselective COX inhibitor indomethacin, TER, or mannitol flux. It also provided no apparent protective effect against the changes in barrier function induced by indomethacin. However, this ex vivo model might be unable to detect additive or synergistic effects of tramadol and COX inhibition that might occur in vivo, so no conclusion can be made regarding the safety profile of tramadol in dogs. This model demonstrates utility, however, for further examination of the effect of pharmacotherapeutics on gastric barrier function.

Footnotes

[a] Cayman Chemical, Ann Arbor, MI
[b] SantaCruz, Dallas, TX

Acknowledgment

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Conflict of Interest: Authors disclose no conflict of interest.

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