Effect of Dosing Interval on the Efficacy of Misoprostol in the Prevention of Aspirin-induced Gastric Injury in the Dog

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EFFECT OF DOSING INTERVAL ON THE EFFICACY OF MISOPROSTOL IN THE PREVENTION OF ASPIRIN-INDUCED GASTRIC INJURY IN THE DOG

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(ABSTRACT)

The effect of reduced frequency of administration of misoprostol on its ability to prevent aspirin-induced gastric injury was evaluated. Twenty-four random-source dogs were divided into 4 groups which received aspirin and misoprostol as follows: Group I, 25 mg/kg aspirin PO TID and placebo PO TID; Group II, 25 mg/kg aspirin PO TID and misoprostol 3 *u*g/kg PO TID; Group III, 25 mg/kg aspirin PO TID, misoprostol 3 *u*g/kg PO BID and placebo PO QD; and Group IV, 25 mg/kg aspirin PO TID, misoprostol 3 *u*g/kg PO QD and placebo PO BID for 28 days. Groups were stratified to contain an equal number of dogs positive or negative for *Helicobacter spp.* based on results of 'CLO test'. Gastroscopy was performed on days –9, 5, 14 and 28. Each region of the stomach was evaluated separately and visible lesions were scored on a scale of 1 (submucosal hemorrhage) to 11 (perforating ulcer). The scores for each region were summed and the median total score for each group at each day and median total score within each group between days was compared using a Kruskal-Wallis test.

No difference in total score was identified between Group I and IV on any day. Median total scores for Groups II and III were significantly($p \leq 0.05$) lower compared to Groups I and IV on day 5. Significant difference was observed on Day 14 between the total score of Group III and Group IV. Group III had a significantly lower score ($p \leq 0.05$) than Groups I, II and IV on day 28. Gastric erosions were present in all groups in the study. This study suggests that misoprostol 3 *u*g/kg PO BID dosing is as effective as misoprostol 3 *u*g/kg PO TID dosing at preventing aspirin-induced gastric injury in this model. However, misoprostol 3 *u*g/kg PO TID dosing was less effective in preventing aspirin-induced gastric injury on days 14 and 28 than in previous studies. The lack of efficacy of TID dosing on days 14 and 28 may be related to higher salicylate concentrations in Group II dogs or individual variation within the small study population.

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Literature Review

Overview of aspirin and other non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs), specifically salicylates, have been used by human beings for their anti-inflammatory, analgesic and anti-pyretic properties for over 3000 years. Salicylate was identified and commercially synthesized in the 1860s, and acetylsalicylate, or aspirin, was developed at the turn of the century.[1] Since that time, many other NSAIDs have been identified and, despite their diverse chemical structures, all are proposed to have the same general mechanism of action. In 1971,Vane et. al. demonstrated that indomethacin, aspirin and sodium salicylate decreased the production of prostaglandins from isolated perfused guinea pig lung in a dose-dependent manner. These results suggested that NSAIDs exert their effects by inhibition of prostaglandin synthesis, elucidating the now-accepted mechanism of action of NSAIDs.[2]

In 1976, a 71 kiloDalton (kDa) membrane bound, glycoprotein enzyme which catalyzes the production of prostaglandins from arachidonic acid was isolated and named cyclo-oxygenase (COX).[3] Cyclo-oxygenase is part of a bifunctional enzyme called prostaglandin H synthase, which catalyzes the first step in converting arachadonic acid to eicosinoids, a class of biologically active lipid molecules that includes prostaglandins, thromboxanes and leukotrienes.[4] Arachidonic acid is a 20 carbon fatty acid that is usually covalently bonded in its ester form in the phospholipid portion of most cell membranes.[5] Phospholipase A2 is an acyl hydrolase that cleaves arachadonic acid from the cell membrane when it is activated by inflammation.[5] Cyclo-oxygenase produces intermediate cyclic endoperoxidases (prostaglandins (PG) G2 and H2), which are subsequently converted to PGD2 and PGE2, PGF2A, PGI2 and the thromboxanes.[5-7] NSAIDS

specifically inhibit the conversion of arachadonic acid to PGG2, thus stopping further production of prostaglandins and thromboxanes.[5]

Cyclo-oxygenase has at least two distinct isoforms; COX-1, the constitutive isoform, which is present in fixed amounts, and COX-2, the inducible isoform.[1] Cyclo-oxygenase -1 is the isoform that is responsible for physiologic functions, such as vasoregulation and cytoprotection of the gastric mucosa.[1] It is found in the stomach and kidneys, as well as in platelets and endothelium throughout the body.[8] The three-dimensional structure of COX-1 has been well-defined and it is speculated that NSAIDs may inhibit the enzyme's action by excluding arachidonic acid from entering the active site in the interior of the enzyme.[9] Cyclo-oxygenase-2 is encoded in a different gene than COX-1.[10-13] and its expression is induced by a number of pro-inflammatory mediators, including cytokines.[14-16] It has been identified in monocytes, fibroblasts, synoviocytes and chondrocytes associated with inflammation.[4,8] The three dimensional structure of COX-2 has been recently described and is similar to that of COX-1.[17] Selectivity among NSAIDs for either enzyme is thought to be conferred by differences in the morphology of the channel which contains the active site of the enzyme.[17]

Aspirin has been shown to inhibit COX by irreversible acetylation of the serine-530 site at the apex of the enzyme, preventing the passage of arachidonic acid into the hydrophobic active site.[18] The serine-530 site is not part of the active site of the enzyme, as its replacement does not alter the effectiveness of the enzyme.[19] In early studies on NSAIDs, salicylate was one order less effective than aspirin when its activity on COX was compared using isolated perfused guinea pig lung, [2] yet its clinical effect is comparable.[19] These initial studies were likely evaluating the effect of these NSAIDs on COX-1, the physiologic form.[19] Aspirin has been demonstrated to have a relatively high affinity for COX-1 over COX-2 (166:1).[20] When aspirin is compared to salicylate for COX-2 activity, the values are comparable, which correlates well to the clinical effects.[19]

Non-steroidal anti-inflammatory agents exert some of their effects independent of inhibition of prostaglandin synthesis.[21] Alternative mechanisms of action proposed for NSAIDs include uncoupling of oxidative phosphorylation, free-radical scavenging, inhibition of leukocyte migration into tissues, inhibition of kinin release, facilitation of cAMP mediated membrane stabilization and effects on metabolism of cartilage and bone.[5,6,21,22] Non-steroidal anti-inflammatory drugs, when administered intrathecally at doses too low to be anti-inflammatory, have been shown to be analgesic, suggesting a central mechanism of action.[8] This effect appears most active when pain receptors have been hypersensitized due to inflammation. The effect may be mediated by inhibition of a spinal COX pathway, or may involve higher centers.[8]

Pharmacologic profile of aspirin

After oral administration, aspirin is absorbed from the stomach and proximal small intestine of dogs and cats.[23] Absorption of aspirin from the stomach is dependent on the drug formulation, gastric emptying time, stomach content and pH of gastric fluids and concurrent drug administration.[24] Once in the stomach, aspirin becomes ionized inthe acidic gastric environment and enters gastric mucosal cells. Inside the epithelial cells, aspirin may become protonated, and remained trapped within the cell.[25] Once absorbed, aspirin is transported in blood bound to plasma proteins.[26] Plasma elimination half-life of aspirin is 6.8 hours in dogs.[27] Aspirin is thenmetabolized in the liver by conjugation with glycine and glucoronic acid. Aspirin and its metabolites are excreted by the kidneys; some pH dependent tubular resorption of salicylates occurs.[24] Salicylates have a longer half life in neonates due the decreased activity of the microsomal enzymes necessary to conjugate and excrete salicylates.[28] By 30 days of age, the plasma elimination half- life is comparable to adult levels.[28] Plain aspirin has been demonstrated to be more irritating to the canine gastric mucosa than buffered or

enteric -coated aspirin, but enteric -coated aspirin also produces the most inconsistent serum salicylate concentrations.[29]

Serum salicylate concentrations between 5 and 30 mg/dL have been demonstrated be therapeutic in the dog.[30,31] An oral dosage of 25mg/kg TID has been recommended to achieve this therapeutic serum concentration. [31] When administered to dogs, plain and buffered aspirin at 25mg/kg PO should achieve therapeutic serum concentrations in 2 hours, with peak serum concentration 4 hours after initial dosing. [29] In contrast, therapeutic serum salicylate concentrations in dogs are not obtained until 50 hours after a single administration of 10 mg/kg of plain aspirin, and peak serum salicylate concentrations remain at the low end of the therapeutic range at that dose.[29] Dosages as low as 3 mg/kg selectively inhibit platelet thromboxane A2 without effecting endothelial PGI2.

Epidemiology of aspirin and other NSAID usage

The use of NSAIDs in people increases with age, primarily due to symptoms associated with chronic musculoskeletal disease.[32] Although epidemiologic data regarding NSAID usage in dogs has not been evaluated, it is likely to show a similar distribution. Osteoarthritis may be the most common reason for NSAID administration in dogs. Clinical effects attributed to aspirin include reduction of fever, reduction in vascular dilation, and alleviation of mild to moderate somatic pain.[6] Indications for its use include post-operative analgesia, osteoarthritis [33], meningitis, soft tissue swelling, heartworm disease[24], uveitis [6,34], endotoxic shock, [23] and prevention of thromboembolic disease.[24]

Adverse effects of aspirin and other NSAIDs: overview

NSAIDs are associated with a number of adverse effects, of which gastro-intestinal side effects are considered to be the most significant in people, resulting in discontinuation of therapy, morbidity and co-treatment with other medications.[32] In one of the most recent reviews of NSAID usage in humans, NSAIDs were shown to increase the risk of gastric ulceration 3-5 fold, and it has been estimated

that 15-35% of all gastric ulcers is due to NSAID administration.[32] Five to 15% of patients with rheumatoid arthritis can be expected to discontinue NSAID therapy due to gastrointestinal side effects.[25] In a typical clinical trial of NSAID usage in which patients were randomized to receive the NSAID diclofenac or placebo, 17% of patients receiving diclofenac reported gastrointestinal side effects, including epigastric pain and indigestion, within 4 weeks of starting therapy, compared to 0.8% initially. Seven percent of patients withdrew from the trial because of these side effects.[32] According to data obtained from the Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS), the mortality rate associated with NSAID-related gastrointestinal effects is 0.22% per year, with an annual relative risk of 4.21 compared to persons not using NSAIDs.[25] The mortality rate among people hospitalized for NSAID-related gastrointestinal hemorrhage is 5-10%.[25] Although this risk seems small for individuals, as a large population of patients is exposed to NSAIDs over extended periods of time, the lifetime risk in individual humans is significant.

Gastrointestinal side effects of NSAID usage described in people include dyspepsia, epigastric pain, and gastric ulceration resulting in perforation and death.[25,35] In addition, other gastrointestinal side effects of NSAIDs have been reported. NSAIDs have adversely affected the small intestine causing inflammation with associated blood and protein loss, intestinal stricture, ulceration, perforation and diarrhea.[25,32] Large bowel perforation and hemorrhage has also been described.[32]

Clinical signs of NSAID toxicosis other than gastrointestinal irritation have been described at plasma concentrations greater then 30 mg/dl and include CNS stimulation, hyperventilation with respiratory alkalosis, hypoglycemia, hepatic necrosis, hemorrhage and renal failure.[26] Other side effects of NSAIDs reported in human beings include cutaneous toxicity, pneumonitis, headaches and aseptic meningitis.[32] With chronic administration, weight loss, anemia and depression may develop. In rare cases, bone marrow suppression has been described.[26]

Salicylates have caused reproductive anomalies, fetal resorption and still-birth in laboratory animals.[26]

Non-steroidal anti-inflammatory drugs exert some adverse effects on the kidneys via reduction of vasodilatory renal prostaglandins such as PGE2 and PGI2.[8,36] The kidneys' reliance on vasoactive effects of prostaglandins increases during conditions of hemodynamic compromise, including dehydration, heart failure, hemorrhage, anesthesia, liver disease and renal disease.[8,32] Administration of NSAIDs under those conditions can result in ischemic injury and acute renal failure, which is usually reversible with appropriate therapy and withdrawal of NSAIDs. The long-term effects of NSAID administration on renal function is uncertain; there is some evidence to support their role in the development of papillary necrosis and tubulointerstitial nephropathy, but a causal relationship, particularly in veterinary patients, has not been established.[8] There is some evidence to support that this effect on renal function may vary with specific NSAIDs, but all NSAIDs to some degree decrease renal prostaglandin production, and can decrease renal function in conditions associated with decreased circulating volume.[32]

Gastric defense and the pathophysiology of NSAID-induced gastric injury

The stomach is protected from the harmful effects of intraluminal contents by a number of mechanisms. The development of gastritis and ulceration requires a breakdown or defect in gastric mucosal defense. Many of the normal constituents of gastric defense appear to require prostaglandins, which may explain in part why NSAIDS are potent gastric irritants. Experimental studies have elucidated many of these mechanisms of gastric defense and documented the role of aspirin and prostaglandins in the development of gastric injury.

Several elements have been defined which constitute the normal defense mechanisms of the gastric mucosa. The stomach is coated by a mucus gel layer, which provides a protective barrier from gastric acid.[8] The mucus gel layer is rich in bicarbonate secreted by the surface epithelial cells of the mucosa, which

creates a pH barrier to acid penetration.[8] In addition, the hydrophobic nature of the gel layer prevents the back diffusion of acid from the lumen to the mucosa.[8] Mucosal tight junctions at the apical surface of the chief cells may be animportant barrier to back-diffusion of acid.[37] Adequate mucosal blood flow is required for production of bicarbonate and mucus, rapid removal of back-diffused acid and for cellular repair.[8] The gastric epithelium has the ability to migrate and divide for quick repair of mucosal injury.[8]

In order for ulceration to occur, a breakdown in the normal mucosal defenses of the gastric epithelium must occur. Five pathophysiologic events have been described which can disrupt this barrier: (1) back diffusion of H+ into the gastric mucosa; (2) failure to buffer H+ entering the surface epithelium; (3) local mucosal ischemia; (4) failure of normal secretion of bicarbonate and mucus; and (5) accelerated cell shedding or decreased rate of cell renewal.[38] Breakdown of the gastric mucosal barrier can be identified experimentally by loss of H+ from the stomach lumen, loss of negative transmucosal potential difference and increases in Na+, CL- and K+ in the gastric fluid.[38,39] Effects on the gastric mucosa associated with loss of mucosal integrity and back-diffusion of acid include release of histamine from mast cells, cholinergic stimulation of acid and pepsin release, vasodilation, edema and protein loss from increased capillary permeability and hemorrhage into the gastric lumen.[40] Agents that have been shown to break down the mucosal barrier include ethanol, bile and aspirin.[38] Agents that break down the gastric mucosal barrier also cause a reduction in electrical potential across the mucosa, which may be the most sensitive indicator of mucosal injury.[38] Once the mucosal barrier is compromised, injury can occur from endogenous factors, such as acid, pepsin and bile salts, as well as exogenous factors, such as NSAIDs and ethanol.[25]

The role of prostaglandins in gastric mucosal defense

Prostaglandins are produced by the action of cyclo-oxygenase on arachadonic acid derived from cell membranes by the activity of phospholipase

A2. In general, prostaglandins react with specific receptors, and regulate intracellular levels of cAMP. In turn, intracellular cAMP concentrations affect the activation of various cellular protein kinases.[6] Physiologic effects of prostaglandins are wide-spread. ProstaglandinE2 is found in virtually every tissue in the body.[41] Prostaglandins of the E, F and I series are produced throughout the gastrointestinal tract.[42] Prostaglandin E2 is the predominant form in the human, feline and canine gastric mucosa. Macrophages and capillary endothelial cells are the major producers of PGE2 in the canine gastric mucosa.[43]

Prostaglandins are generally thought to be pro-inflammatory compounds. Classic functions attributed to prostaglandins include mediation of fever via IL1, inhibition of suppressor T Cell function, and synergistic mediation of pain and vasodilation with bradykinins.[21] Prostaglandins do not cause pain themselves, but sensitize the nerve endings to pain stimuli.[8] Anti-inflammatory characteristics attributed to prostaglandins include inhibition of inflammation associated with adjuvant arthritis and murine nephritis.[21] Early clinical studies suggest the PGE may be useful in reducing inflammation associated with hepatitis, renal transplantation and immune-mediated arthritis.[21]

Release of prostaglandins in the gastrointestinal tract appears to be mediated by neurohormonal stimulation of digestive glands, wall tension, local autocrine stimulation and the presence of irritants in the gastric lumen.[42] Reactive oxygen metabolites, which may be produced in response to ischemia or inflammation, have been shown to stimulate prostaglandin E2 production in canine gastric mucosa.[44] Prostaglandins are rapidly metabolized by the enzymes 15-OH-prostaglandin dehydrogenase and 13, 14-reductase present in the gastrointestinal mucosa, liver and lung.[45]

Cytoprotectant effects attributed to prostaglandins in the gastro-intestinal tract include stimulation of mucus production, stimulation of bicarbonate secretion, inhibition of acid secretion, improved mucosal blood flow, and stimulation of epithelial cell turnover and repair.[46] Numerous experimental studies have

elucidated the role of prostaglandins in almost every aspect of normal gastric mucosal defense.

Prostaglandins have been shown to prevent histologic changes associated with high intraluminal acid concentrations in canine chambered stomach preparations.[47] The increase in pH of the mucus layer suggests that stimulation of bicarbonate secretion may be a component of this effect. Sixteen, 16-dimethyl prostaglandin has been demonstrated to stimulate production of bicarbonate secretion in dogs in vivo, but not in vitro, suggesting that an intact blood supply is required.[48] Topical application of PGE2 to a canine chambered stomach preparation results in increased production of bicarbonate in a dose-dependent fashion.[49] This effect on bicarbonate secretion is prevented by the addition of atropine and tetrodotoxin, suggesting that the effect is mediated through acetylcholine.[49]

Synthesis and release of mucin and surfactant phospholipids by canine gastric mucous cells is important to maintain the hydrophobicity of the gastric mucosa.[50] Histopathologic staining studies of canine gastric mucosa have suggested that the hydrophobicity of the canine gastric mucosa is maintained by the phospholipid component of the mucus gel layer.[51] Production of mucus and improvement in surface hydrophobicity by in vitro canine mucous cells in response to stimulation with prostaglandin E2 has been demonstrated by histologic evaluation[51] as well as contact angle evaluation, an indirect measure of mucosal hydrophobicity.[52] Production of surface-active phospholipids by primary cultures of canine gastric mucous cells is significantly increased by the addition of PGE2.[50]

Prsotaglandin –mediated regulation of gastric acid secretion plays a role in gastric mucosal protection; however, the exact mechanism by which prostaglandins influence gastric acid secretion is poorly defined. Both PGE2 and PG12 produce dose-dependent inhibition of acid and pepsin secretion in canine gastric mucosa stimulated by pentagastrin.[53] The exact mediators of this stimulation are not known, Pre-treatment of isolated canine gastric mucosal cells

with 5% ethanol has been shown to result in prostaglandin production by the portion of the stomach rich in mast cells, suggesting a role for mast cells in the gastric mucosa in adaptive cytoprotection to irritant stimuli.[54] Mast cells and histamine release may also play a role in prostaglandin-mediated suppression of acid secretion, as histamine release by canine fundic mast cells is inhibited by PGE2 in vitro.[55] However, the role of histamine in prostaglandin-mediated acid secretion is contraversial, as neitherPGE2 or PGI2 at antisecretory doses affect pentagastrin-stimulated gastric histamine release.[56] Infusions of PGE2 and PGI2 at antisecretory doses have been shown to increase gastric vasodilation in anesthetized healthy dogs, so changes in local blood flow may be a factor.[56] Regardless of the mechanism of action, it is thought that prostaglandin protection from the toxic effects of aspirin may be partially related to acid suppression, as prostaglandins reverse changes in transmucosal potential difference, short circuit current and net sodium transport caused by aspirin in a canine model within 40 minutes of topical application [57] and the extent of aspirin-induced mucosal injury to canine gastric mucosa has been demonstrated to vary based on the pH of luminal fluid.[58]

Prostaglandins have been shown to protect the gastric mucosa against a number of harmful irritants, including aspirin. Addition of 16, 16-dimethyl prostaglandin to canine gastric mucosa infused with acidified aspirin results in maintenance of surface hydrophobicity and reduced exfoliation of surface epithelium, but does not attenuate cellular damage to the surface epithelium.[59] This indicates that although the mucous gel layer can not prevent the effects of aspirin on the mucosa, the ability to support the hydrophobic mucus gel layer may partially explain its ability to limit aspirin-induced gastric injury. Sixteen, 16-dimethyl prostaglandin E2 has been demonstrated to enhance the recovery of surface hydrophobicity and transmucosal potential difference of canine gastric mucosa exposed to acidified aspirin.[60] In 1983, Konturek et. al. demonstrated that bicarbonate secretion from oxyntic, antral and duodenal mucosal cells of conscious dogs can be stimulated by prostaglandins of the E and F series,

suggesting the prostaglandins have a physiologic role in alkaline secretion.[61] Increased production of mucus has been identified in canine chambered stomach models pretreated with 16, 16-dimethyl prostaglandin E2, but this effect did not reduce back diffusion of hydrogen ions and changes in transmucosal potential difference associated with topical application of aspirin.[62]

Mechanism of NSAID-induced gastric injury

Several mechanisms by which non-steroidal anti-inflammatory drugs exert their adverse effects on the gastrointestinal system have been described. Many, although not all, of these effects involve the ability of NSAIDs to reduce local production of prostaglandins, thus overwhelming normal gastric defense. Many of the studies regarding aspirin and prostaglandin production were performed using a canine model and will be described below.

NSAIDs can exert a direct toxic effect on the gastric mucosa. Some NSAIDs are weak acids, which are non-ionized and lipophilic at intra-luminal gastric pH and readily transported into epithelial cells, where the increase in pH causes them to become ionized and trapped within the cell, resulting in toxicity.[25] Aspirin is the prototypical drug with this mechanism of GI injury. NSAIDs have been demonstrated to decrease gastric mucus hydrophobicity, resulting in increased susceptibility of the gastric mucosa to injury from gastric acid and pepsin.[63] Active metabolites of some NSAIDs may be excreted in bile and cause indirect injury to the gastric mucosa via gastro-duodenal reflux.[25]

In addition to direct topical injury, aspirin and other NSAIDs are thought to exert much of their effect on gastric mucosa via reduction of mucosal prostaglandin production. The use of enteric -coated aspirin[64], parenteral or rectal administration of NSAIDs has not been effective in reducing gastric injury associated with NSAID administration in people.[25] This suggests that the systemic effect of decreased prostaglandin production plays a larger role than topical injury in the development of NSAID-induced gastric injury.[25] Aspirin has been demonstrated to reduce the production of PGE2 and PGI2 in rat stomachs

by 75%-94% in a dose-dependant manner.[65] This reduction in prostaglandin synthesis was associated with increased mean area of gastric ulceration.[65]

The adverse effects of aspirin on the canine gastric mucosa have been well documented. Application of acidified aspirin to in vitro models of canine gastric mucosa results in exfoliation of surface epithelium, reduced surface hydrophobicity, and reduced transmucosal potential difference which recovered after removal of aspirin.[59,60] Aspirin administered at 3-50 mg/kg IV to dogs with chambered stomachs resulted in increases in mucosal blood flow and acid secretion, but these effects appeared to occur independently, suggesting that they are not causally related.[66] Topical application of aspirin to isolated canine stomachs results in focal reduction in gastric mucosal blood flow as determined by hydrogen gas clearance[67] and [14C]aminopyrine clearance.[68,69] Spontaneous return to normal blood flow occurs when aspirin is removed from the mucosal surface.[67] In contrast, other studies have demonstrated no change or increase in mucosal blood flow measured by radiolabelled microspheres associated with the development of gross, aspirin induced lesions.[70,71] Ultrastructural effects of aspirin on rat gastric mucosa include clumping of nuclear chromatin, mitocondrial swelling, and swelling and rupture of the apical membrane.[72]

Non-steroidal anti-inflammatory drugs have been shown to adversely affect gastric mucosal defenses by measured changes in transmucosal electrical potential difference, electrical resistance, ion flux and bicarbonate secretion.[73] Aspirin has been shown to decrease transmucosal potential difference and short circuit current and net sodium transport in canine gastric mucosa.[57] Aspirin can cause several morphologic abnormalities of the canine gastric mucosal tight junctions including discontinuation of the apical occluding complex, hyperplasia of the tight junction and variability in the number of tight junction strands.[74] One study demonstrated as much as 50-60% of the superficial mucosa may be injured by application of aspirin.

Aspirin may also alter the composition of gastric mucinous secretion in dogs; canine gastric mucosa treated with aspirin showed decreased

concentrations of major carbohydrate components and amino acids in gastric secretions compared to controls.[75] Luminal concentrations of Na+, K+, Ca++ and Mg++ are increased and H+ concentration decreased in Heidenhain pouches (vagally-denervated in vitro canine stomach models)[69,76] and isolated canine gastric mucosa [77]after topical application of aspirin, suggesting increased back diffusion of H+.[69] The changes in luminal ion concentrations are associated with a decrease in mucosal ATP, suggesting inhibition of ion transport and subsequent increase in mucosal permeability as a mechanism for aspirin-induced gastric injury.[77]

Reduction of local prostaglandin production in the gastric mucosa of dogs associated with concurrent treatment with NSAIDs has been documented both directly, by measuring prostaglandins, and indirectly, as described above, by evaluating the effects of NSAIDs on prostaglandin-dependent processes. Dose dependent reduction in PGE2 levels in interstitial fluid from the canine stomach and in biopsy specimens of the fundus and antrum has been demonstrated in response to indomethacin.[78] Aspirin has been shown to reduce the concentration of PGF2a and PGE2 as measured by radioimmunoassay in samples of mucosa obtained from vagally denervated gastric fundic pouches in healthy dogs.[79]

The difference in relative selectivity for COX iso-forms by various NSAIDs may explain the variability in side effects associated with individual NSAIDs. Drugs that have a relatively high affinity for COX-2 over COX-1 may havethe same anti-inflammatory properties with fewer adverse effects than those with a higher COX-1 affinity.[4] This is supported by the relatively high incidence of gastrointestinal side effects with drugs that have a high COX-1 affinity (aspirin, indomethacin, piroxicam), when compared to drugs with a more favorable COX-2/COX-1 ratio.[1,80] When data regarding COX-1 and COX-2 activity for a variety of NSAIDs (obtained using either bovine aortic endothelial cell/endotoxin-stimulated mouse macrophage or unstimulated versus lipopolysaccharide-stimulated guinea pig macrophages) is compared to data regarding the incidence of associated GI side effects, an increased COX-2 to COX-1 ratio was associated

with decreased GI side effects [4]. Meta-analysis of 12 controlled epidemiologic studies that reported a relative risk between NSAID usage and peptic ulcer disease in human patients from 1985 to 1994 showed that different NSAIDs are associated with different relative risk of developing gastrointestinal side effects .[81] Azapropazone, tolmetin, ketoprofen and piroxicam were associated with the highest risk; aspirin was considered of intermediate risk.[81]

Recent evidence has supported the claim that other mechanisms may be involved in NSAID-induced gastric injury besides inhibition of prostaglandin synthesis. Cyclo-oxygenase-1 deficient mice do not develop spontaneous gastric ulceration, suggesting that inhibition of COX alone may not explain the development of gastric lesions in animal receiving NSAIDs.[25] Recent studies have implicated neutrophil activation as an important component in NSAID induced gastric injury.[82] Adherence of neutrophils may be related to the loss of vasodilatory prostaglandins in the gastric mucosa, and resultant ischemia and platelet aggregation.[8,83]

Several papers have documented the effects of NSAIDs on the gastrointestinal tract of humans.[84-86] Gastrointestinal clinical signs in humans have been shown to be a poor indicator of the presence of erosions or ulcerations seen with an endoscope, with up to 25% of NSAID-induced gastric ulcers being asymptomatic, and 50% not having classical clinical signs. [25,32,35] In endoscopic studies, all areas of the stomach are effected by NSAID administration; however, the gastric antrum appears to be the most frequently and severely affected area in humans.[87]

Interactions between Helicobacter spp. and NSAIDs

Helicobacter spp. infection and NSAID usage are the two major, independent variables associated with the development of gastric ulceration in human patients.[32] Most studies have not demonstrated increased risk of developing a NSAID ulcer in patients with concurrent *Helicobacter ssp*. Infection.[32,88-91] However, patients with both factors have a higher risk of

gastric ulceration than those with neither factor.[32] Some recent studies have raised the question of synergy between the two factors. Porro et. al [92]performed a prospective study evaluating rates of ulcer healing in patients receiving NSAIDs with concurrent *Helicobacter pylori* infection. Although the presence of *Helicobacter pylori* did not affect the rate of ulcer healing, the rate of ulcer recurrence was higher inpatients who were still infected when compared to rates in those patients in whom the organism had been eradicated. The difference, however, was not statistically significant. Chan et. al [93] found that gastroduodenal ulcers developed in 26% of naproxen users with concurrent *Helicobacter pylori* infection, compared to 7% of those in whom the organism had been eliminated using a standard treatment regimen. However, interpretation of these results is confounded by the presence of bismuth in the drug regimen, which may stimulate prostaglandin synthesis in the gastric mucosa.[25] In a large study of 285 patients, Hawkey et. al [94] prospectively evaluated the response of NSAID using patients with recurrent ulcers to two different *Helicobacter pylori* treatment regimens. Eradication of *Helicobacter pylori* did not affect the rate of recurrence of ulcers in these patients. While the data is conflicting, concurrent infection with *Helicobacter pylori* seems to affect the risk of NSAID-induced gastric injury minimally, if at all.[25]

Adverse effects of aspirin and other NSAIDs: veterinary studies

The incidence of gastrointestinal side effects associated with NSAIDs in dogs is difficult to assess. In one review of telephone calls to the Georgia Animal Poison Information Center, 190 inquiries regarding NSAID toxicosis in dogs were received over a 19-month period.[95] Clinical signs reported were most often related to the gastrointestinal tract; including vomiting, diarrhea, hematochezia, regurgitation and abdominal pain. Over 90% of the calls regarded accidental ingestion of NSAIDs, so no conclusions can be drawn about those patients intentionally receiving NSAIDs as therapy. In a retrospective review of 43 dogs with gastroduodenal ulceration evaluated at the University of Tennessee over a

10-year period, treatment with NSAIDs was the most common predisposing factor to ulcer development, although 8 of 10 dogs with NSAID exposure also had other risk factors.[96]

No large study of dogs receiving NSAIDs for medical problems and the incidence and clinical signs of gastrointestinal side effects is available in the veterinary literature. Several individual cases have been reported involving naproxen[97,98], aspirin[99], piroxicam[100], indomethacin[101] , flunixin meglumine[102]and ibuprofen.[103] In one retrospective evaluation of canine cases of NSAID-induced gastric ulceration seen at the Animal Medical Center in New York City between 1984 and 1987, the clinical signs and outcome of seven cases were reported by Wallace et. al. [104] All seven dogs were receiving NSAIDs for orthopedic problems. Clinical signs included melena (6 dogs), vomiting (5 dogs), weakness and collapse (4 dogs). All dogs were anemic at presentation. Non-steroidal anti-inflammatory drugs associated with disease in these dogs included aspirin (4 dogs), naproxen (1 dog), phenylbutazone (1 dog), ibuprofen (2 dogs) and meclofenamic acid (1 dog). Upper GI endoscopy was performed on five dogs. Lesions included multiple bleeding ulcers (2/5), 2 deep crater-like ulcerations (1/5), small non-bleeding ulcer (1/5) and multiple petechiations and submucosal hemorrhages (1/5). All dogs recovered with appropriate supportive care.

Despite the paucity of clinical reports on the subject, several experimental studies have been performed to evaluate the gastrointestinal effects of NSAIDS in dogs. In 1964, Hurley et. al. [105] demonstrated consistent gastric lesions in medium-sized dogs given 600 mg of plain aspirin or buffered aspirin twice daily as early as four hours after initiating medication. Lesions consisted of deep gastric erosions and most resolved spontaneously between 3 to 13 days after initiation of the study. Similar lesions were not observed in dogs that received an effervescent buffered aspirin solution at the same dosage for 11 days.

Taylor et. al. [106] gave 4 dogs aspirin in daily doses ranging from 45-64 mg/kg per day. Dogs were sacrificed after different lengths of treatment and the gastrointestinal tract evaluated for hemorrhage. Hemorrhage was noted in the 2

dogs sacrificed at 12 and 18 days of treatment, but not in those dogs sacrificed later. In another study, Phillips [107] demonstrated intestinal microbleeding in dogs by radiolabelled iron within 7 days of administration of plain aspirin at doses between 32 and 98 mg/kg BID. Although the location of hemorrhage along the gastrointestinal tract could not be identified, the amount of bleeding appeared dose-dependent.

In 1996, Forsyth et. al. [108] divided healthy dogs into five groups who received either clinically recommended doses of ketoprofen, copper-indomethacin, prednisolone/cinchophen, aspirin or gelatin for seven days. Endoscopic lesions were noted in all groups; the dogs receiving gelatin had significantly less severe lesions than the other groups. Those dogs receiving aspirin had significantly higher lesion scores. No clinical signs were noted in any dogs in the study. The incidence of lesions in dogs receiving NSAIDs in this study was 88%.

In 1998, another study by Forsyth [109] evaluated the gastroduodenal mucosa of 24 dogs after 7 and 28 days of oral NSAID administration. The dogs were divided into 4 groups who received respectively carprofen, ketoprofen, meloxicam or gelatin placebo. Mild to moderate gastroduodenal lesions were observed in 17 of 28 dogs. No statistical difference in lesion scores between groups was detected and no dogs showed clinical signs related to NSAID administration.

In 1999, Reimer et. al.[110] evaluated the effects of aspirin 16.5 mg/kg PO BID, carprofen 2.2 mg/kg PO BID and etodolac 12.8 mg/kg PO QD on healthy dogs. All drugs were administered for a total of 28 days. Upper gastrointestinal endoscopy was performed on days 0,5,14 and 28 and the severity of lesions in the stomach and proximal duodenum was compared between groups. The dogs receiving aspirin had significantly higher lesion scores on days 5, 14, and 28, than those dogs receiving carprofen and etodolac. No difference in gastrointestinal clinical signs, which included mild vomiting and diarrhea, was noted in any group.

In a clinical review of 43 cases of gastric ulceration in dogs, the pyloric antrum was the most common location for ulceration associated with

administration of NSAIDs.[96] Non-steroidal anti-inflammatory drugs involved included naproxen, indomethacin, flunixin, piroxicam and aspirin. In other endoscopic studies of the effect of NSAIDs on the gastric mucosa of dogs, the pyloric antrum also appears to be the most severely affected region by flunixin[111], flunixin plus prednisone[111], aspirin[29,112], ketoprofen[109] and meloxicam [109]. In contrast, Reimer et. al. [110]and Johnston et. al. [113] failed to demonstrate any significant difference in lesion score between gastric regions in dogs receiving aspirin, aspirin with misoprostol, etodolac or carprofen for 28 days. All gastric regions were affected equally in both studies. However, significantly fewer lesions were observed in the duodenum compared to the individual gastric regions in both studies.

In human medicine, enteric-coated and buffered aspirin have been recommended to reduce the incidence of gastrointestinal side effects of NSAIDs.[64,114] The effect of different formulations of aspirin on the development of gastrointestinal lesions in dogs has been evaluated by Lipowitz et. al.[29] They divided 36 dogs into six equal groups who received the following treatments at 8 hour intervals for 7 treatments; Group 1, aspirin 25 mg/kg; Group 2, aspirin 10 mg/kg; Group 3, buffered aspirin 25 mg/kg; Group 4, enteric-coated aspirin, 25 mg/kg; Group 5, buffered aspirin (different brand) at 25 mg/kg; and Group 6, placebo. Administration of aspirin-containing products resulted in therapeutic concentrations of aspirin in all dogs except Group 2. The greatest fluctuation in serum salicylate concentrations was found in Group 4. Seventy-two hours after initiation of treatments, upper GI endoscopy was performed on dogs from Groups 1,3,4, and 5. Gastric lesions ranging from mild petechiation to linear hemorrhage were identified only in those dogs receiving 25 mg/kg of plain aspirin (Group 1). Although this study was of short duration with a small number of dogs, enteric-coated and buffered aspirin appeared to produce fewer endoscopically-detectable gastric lesions than plain aspirin.

Treatment of NSAID-induced gastric injury

Much information regarding ulcer therapy in veterinary medicine has been extrapolated from human medicine.[46] Several classes of drugs have been used for this purpose and will be briefly reviewed here.

Antacids

Antacids, such as magnesium hydroxide, aluminum hydroxide and calcium carbonate, have been effective in the treatment of gastric ulcers in humans, but have not been evaluated in companion animals.[46] Although these drugs have been demonstrated to be effective in treating ulcers in humans, they have been largely replaced by newer medications because they require frequent administration to be effective, which makes their use impractical in dogs and cats, and they can not be administered parenterally.[46] Although the effectiveness of antacids in neutralizing acid is thought to be the primary mechanism of gastric healing, there is some evidence suggesting antacids may stimulate local production of prostaglandins in the GI mucosa as well.[46]

H2 Receptor Antagonists

Parietal cells are stimulated to produce gastric acid via three receptors; the histamine (H2) receptor, the gastrin receptor and the acetylcholine receptor. Although there is interaction between all three receptor types, blocking any one receptor results in a significant decrease in gastric acid production.[46] Histamine-2 receptor antagonists currently available include cimetidine, rantidinine, famotidine, and nazatidine. Ranitidine and famotidine are more potent and slightly more expensive than cimetidine. At equivalent dose, neither is more effective in treating ulcers in human beings than cimetidine.[46] Currently, none of these drugs is approved for veterinary use. Experimental studies have demonstrated the efficacy of cimetidine in reducing acid secretion in the dog.[46]

Omeprazole

Omeprazole reduces gastric acid production by blocking the luminal H+/K+ ATPase on the parietal cell. It has a longer duration of action and is more potent

than cimetidine in dogs.[46] Omeprazole has been shown to be equal for healing ulcers when compared to H2 antagonists in human beings.[46]

Sucralfate

Clinical studies have demonstrated that sucralfate is equally effective as cimetidine in treating ulcers in humans.[46] Sucralfate is broken down in the stomach by gastric acid into sucrose sulfate and its aluminum salt. Sucralfate demonstrates affinity for damaged tissue based on the attraction of the negatively charged sulfate groups to the positively charged exposed tissue, binding to ulcerated areas and protecting them from acid and pepsin.[46] Sucralfate has also been demonstrated to stimulate prostaglandin production from the gastric mucosa, increase the production and viscosity of mucus, and increase bicarbonate secretion.[46]

Despite the success of these classes of drugs in treating NSAID-induced gastric ulceration, there is little evidence in the human medical literature to support their efficacy in preventing the development of gastric complications of NSAID therapy. A large controlled study by Agrawal et. al demonstrated no benefit of sucralfate in preventing gastric ulceration in patients with osteoarthritis taking NSAIDs.[115] Two large, placebo-controlled studies showed that ranitidine reduced the risk of duodenal ulceration associated with NSAID usage, but did not prevent gastric injury.[116] High doses of famotidine were effective, but only minimally and the cost of therapy was prohibitive.[117] Omeprazole has shown some recent promise in the prevention of NSAID-induced gastric injury in people.[118] None of these drugs has been evaluated in clinical veterinary patients.

Misoprostol

Misoprostol is a synthetic prostaglandin E1 analogue.[119] It is the first synthetic prostaglandin analogue evaluated for the treatment of gastric ulceration, and the only one approved by the Food and Drug Administration for the prevention of NSAID-induced gastric ulcers in humans.[41] The effect of prostaglandins on gastric acid production has been documented for years. However, orally

administered prostaglandins are rapidly metabolized by the gastrointestinal mucosa, liver and lungs, precluding their pharmacological usefulness.[73] Chemical instability and short duration of action are other factors limiting the usefulness of natural prostaglandins.[41] Synthetic prostaglandins are more potent than naturally occurring prostaglandins, have less side effects, are longer acting and resist rapid metabolism, making them effective with oral administration.[41,73,120]

Misoprostol consists of 4 isomers (SC-30422, SC-30423, SC-30248, and SC-30249), as well as small amounts of impurities (SC-29636, SC-32759, SC-33188).[119] Although studies have demonstrated some biologic activity (reduction in acid secretion) for all the impurities and isomers, only SC-30249 has similar biologic activity to all four isomers of misoprostol together.[119] Other metabolites of misoprostol include PGF1 and its derivatives.[41] Misoprostol is rapidly de-esterified to its free acid counterpart.[121] The free acid is then converted to dinor and tetranor metabolities via B-oxidation of the carboxyl side chain.[122] Studies in isolated histamine-stimulated canine parietal cells have shown that the acid counterpart is as effective at suppressing acid secretion as misoprostol itself, suggesting misoprostol may act as a pro-drug.[122]. The dinor metabolite is a very weak inhibitor of acid secretion, and the tetranor metabolite has no demonstrable effect against acid secretion.[122]

Pharmacokinetic studies have been performed in dogs and humans using radio-labelled misoprostol. Absorption is complete in both species within 1.5 hours.[121] Peak plasma concentrations of total radioactivity are similar in humans and dogs (2.58 +/- 0.26 ng/ml; 2.24 +/- 0.45 ng/ml respectively) at the same administered dose.[121] Eighty percent of the total radioactivity was excreted in the urine and feces within 24 hours in dogs and humans.[121] The free acid metabolite of misoprostol is 85% bound to albumin in the circulation. This effect is independent of age, concentration and concommittant drug therapy.[41] Serum binding of misoprostol was studied in human subjects with concurrent

administration of aspirin. Aspirin is also extensively bound to serum proteins and might be expected to compete with misoprostol for transport. Serum binding of misoprostol was lowered (by 25%) only when salicylic acid concentration exceeded 300 ug/ml.[121,123] Binding of misoprostol appears to be saturable, reversible and stereospecific.[124,125] High-affinity PGE receptors have been identified on isolated canine parietal cells, which bind misoprostol, but not cimetidine or histamine, and PGI and PGF bind only slightly.[124,125] It has recently been demonstrated that misoprostol activity and receptor binding in canine parietal cell preparations is comparable to that of its acid counterpart; however, when an esterase inhibitor is added to this preparation, the efficacy of misoprostol is markedly reduced, suggesting that the free acid counterpart is the active metabolite of the drug.[126]

Misoprostol: mechanism of action

While the exact mechanism of misoprostol cytoprotection is uncertain, several concepts have been developed to explain its effect. Among the most significant potential mechanisms are maintenance of mucosal bicarbonate and mucus secretion, effects on the gastric mucosal barrier, effects on mucosal blood flow and and effects on cellular migration and proliferation.

Numerous experiments have documented the effectiveness of misoprostol in enhancing gastric mucosal protection. Misoprostol is a potent antisecretogue. Misoprostol inhibits acid production stimulated by coffee and meals[127], as well as basal and nocturnal acid production in healthy humans.[128] In humans, 200 *u*g significantly inhibited meal stimulated acid production for 3 hours, reduced secretion for 5.5 hours and had no effect on acid secretion after 8 hours.[73] In another study, maximal acid secretion after a meal at 60 and 90 minutes was 85% and 75% respectively.[73] In experimental human models, histamine, tetragastrin, betazole, and pentagastrin-induced acid production are suppressed by misoprostol.[129] Experimental studies in dogs have demonstrated that misoprostol is effective at reducing gastric acid secretion stimulated by histamine,

pentagastrin, and beef-meal stimulation at a dose of 3 *u*g/kg. This resulted in inhibition of acid secretion from 30-95% of normal, by both decreasing acid concentration and volume of acid produced.[119] This effect is maximal with intravenous administration, and least with intra-gastric administration. Proteolytic activity was reduced as well. The anti-secretory effect of misoprostol can be demonstrated on isolated rabbit gastric glands stimulated with histamine[119], as well as in isolated canine parietal cells[122], suggesting a direct effect on parietal cells. The potency of the anti-secretory effect was greatest when misoprostol was in direct contact with the gastric mucosa, suggesting local effect and absorption.[119] The anti-secretory effect in rats in response to pentagastrin stimulation was not associated with reduction in mucosal blood flow.[130]

The anti-secretory property of misoprostol is postulated to be at the level of the histamine receptor or the production of cAMP after stimulation of the histamine receptor by inhibiting activation of the histamine-sensitive adenylate cylase.[42,124] This effect is likely mediated by a prostaglandin receptor located on or near the histamine receptor.[119] Misoprostol has demonstrated less incidence of recurrence of ulcers after cessation of therapy when compared to H2 receptor antagonists.[41] This may be related to the lack of increase in gastrin production associated with misoprostol administration[131], which has been demonstrated with H2 receptor antagonists.[41] When serum gastrin concentration was evaluated in dogs via RIA one hour after meal-stimulation, then again one hour after 3*u*g/kg IV infusion of misoprostol, no significant difference in serum gastrin concentrations could be demonstrated, although gastric acid production was reduced by 95%.[119] The dose of misoprostol currently recommended in human beings(200 *u*g) is effective in reducing acid secretion.[73]

The cytoprotectant effects of prostaglandins on the GI tract are effective for a range of noxious factors other than NSAID administration, including chemical and physical insults.[73] The acid-independent cytoprotective effect has been demonstrated by studies using non-acid suppressing doses of prostaglandins[132],

and by demonstrating effectiveness of prostaglandins against noxious agents that do not rely on acid secretion for effect.[133] Intravenous infusion of PGE2 and PGI2 at doses that did not alter gastric acid secretion have been demonstrated to protect against aspirin-induced gastric injury in rats.[65] Misoprostol has been shown to protect against gastric ulceration in numerous rat models at doses 10 fold lower (10-150 *u*g/kg) than the dose required to reduce acid secretion (1000 *u*g/kg) .[119] Misoprostol prevented ethanol-induced gastric injury in rats[134], primates[135], and healthy male humans[136] when compared to placebo at doses lower than antisecretory doses.

The exact mechanism by which misoprostol exerts this cytoprotectant effect is uncertain. Enhancement of local mucus production may explain some of the effect. Misoprostol has been shown to increase gastric mucus production in humans in a dose-dependent manner.[137] In rats, the adherent layer of mucus can be increased up to three-fold in thickness, with 70% of the response noted within 5 minutes.[138] This suggests that misoprostol stimulates the release of pre-formed mucus.[139] This increased mucus production has been postulated to prevent the back-diffusion of hydrogen ions, resulting in mucosal protection.

Support of gastric mucosal turnover and regeneration is also postulated to be a factor in misoprostol cytoprotection. Prostaglandins of the E series, including misoprostol have been demonstrated to significantly increase canine gastric mucosal mass.[140] This effect may be due to increased DNA and cell proliferation, or decreased cell loss. In 1990, Goodlad et. al. evaluated gastric cell migration rates and transit time using radiolabeled (^{3}H-thymidine) autoradiography in dogs that had received 300 *u*g/kg PO QD of misoprostol for 11 weeks. Although the migration rate towards the gastric lumen was significantly increased on all days, the gland length was also increased, resulting in no significant difference in median transit time when compared to control dogs.[141]

Using the same technique, Goodlad et. al.were able to demonstrate that the increase in gastric mass associated with administration of misoprostol is related to increased cell proliferation, rather than decreased cell turnover.[142] Administration

of 300 *u*g/kg/day of misoprostol resulted in overall increased gastric mass, with an increased proportion of mucosal volume occupied by mucus cells, and a decreased volume fraction of parietal cells.[143] In a recent experiment, dogs were given misoprostol at a dose known to increase gastric epithelial cell proliferation, and plasma and tissue levels of numerous gastrointestinal regulatory proteins were evaluated.[144] Misoprostol did not affect plasma concentrations of enteroglucagon, cholecystokinin, insulin and glucose-dependent insulinotrophic peptide, or tissue concentrations of bombesin-like immunoreactivity, gastrin and somatostatin, suggesting the trophic effect of misoprostol on canine gastric mucosa is direct.

The role of misoprostol in enhancing bicarbonate secretion has been evaluated as well. Misoprostol stimulates duodenal production of bicarbonate in rats[145] and humans[146] in a dose-dependent manner. Quantitative microscopic analysis of injury showed that the magnitude of aspirin-induced gastric mucosal injury is pH-dependant, with the most significant lesions at pH 1.[147] Topical application of misoprostol to canine chambered gastric mucosa results in a composite alkaline secretion composed primarily of Na+, Cl-, with smaller amounts of K+ and HCO3-.[148,149]

Increases in mucosal blood flow have been postulated as a mechanism of misoprostol-induced gastric protection. However, several experimental studies have produced contradictory results regarding the role of mucosal blood flow in misoprostol-induced gastric protection. Gastric mucosal blood flow, as measured by C-aminopyrine clearance in dogs, was not significantly altered by intravenous administration of 0.3 *u*g/kg or 1 *u*g/kg of misoprostol.[119] In addition, PGF2A, a potent vasoconstrictor, has been shown to be a mucosal protectant, suggesting the role of increased blood flow as a mechanism of misoprostol-induced mucosal protection may be questionable.[132] In contrast, in an experimental model of isolated versus perfused canine gastric mucosa, pre-treatment with 16,16-di-methyl prostaglandin was shown to protect against injury caused by topical application of aspirin in vivo, but not in vitro, suggesting that blood flow and neural

factors may be required to mediate the effect of prostaglandins.[147] Misoprostol has been shown to cause transient increase in focal mucosal blood flow in dogs, as detected by laser-Doppler flowmetry, but not hydrogen gas clearance.[150]

Topical application of misoprostol to the resting canine gastric mucosa resulted in histologic changes including increased thickness of the mucosa and submucosa, dilated intraglandular regions of the lamina propria, marked subepithelial edema, reduced depth and width of gastric fovea, vasodilation of the vascular channels, reduced height of surface epithelial cells and swelling of the basolateral intercellular spaces.[151] These lesions were considered harmless; the authors postulate that the edema may be secondary to increased mucosal permeability. Proposed mechanisms by which mucosal edema could result in gastroprotection include increasing the distance for absorption of toxic compounds, diluting the concentration of a toxin or disseminating focal accumulations of red blood cells.[151]

Alterations in mucosa structure are unlikely to be a mechanism of action for misoprostol. Quantitative freeze-fracture analysis of rat gastric epithelium showed that mucosal tight junction alterations do not play a role in misoprostol cytoprotection.[152] Fich, et. al. showed that no structural changes in human gastric mucosa or increases in rates of cell turnover were noted with administration of misoprostol, making restoration of mucosal repair an unlikely mechanism of misoprostol activity.[153]

Several experimental studies evaluating the effectiveness of misoprostol in protecting the canine stomach from adverse effects of NSAIDs have been performed. Aspirin will cause a significant, dose-dependant decrease in gastric transmucosal electrical potential in dogs which is attenuated by administration of intra-gastric misoprostol.[119,154] Disruption of the canine gastric mucosa by aspirin, as evidenced by decreased electrical potential difference, and loss of K+ and blood into the gastric lumen, are attenuated by intragastric administration of 1-3 *u*g/kg of misoprostol.[154] DNA release into the gastric lumen was not altered,

suggesting that superficial injury is not altered by administration of misoprostol, and that the clinical effect may be related to protection of deeper tissues.[154]

The exact anatomical location within the layers of the gastric mucosa where prostaglandins such as misoprostol exert their protective effect has been the subject of several studies. Most histologic evaluations of the gastric mucosa of rats and dogs have shown that the PGE2 protective effects against many noxious stimuli are primarily limited to the deeper, glandular layers of the mucosa,[133,134] with superficial gastric injury unaffected by prostaglandins. In contrast, Henegan demonstrated sparing of the superficial layers of perfused canine gastric mucosa (60% intact in stomachs treated with PGE2compared to 23% in controls) as well as deeper layers.[147] Despite the significant histologic reduction of superficial aspirin-induced gastric mucosal injury observed in Henegan's experiment, no accompanying change in transmucosal potential difference was recorded.[147] which is normally observed with prostaglandin cytoprotection. However, this may have been related to the relatively short study time in the latter experiment, suggesting that prostaglandins may reduce superficial injury as well as deeper injury.

Human clinical experience with misoprostol

Misoprostol has been shown to be superior in the prevention of gastric injury in long- and short-term administration of NSAIDS when compared to placebo by intestinal micro-bleeding determinations and endoscopy.[155] This effect has been demonstrated during treatment with aspirin, naproxen, ibuprofen and tolmetin, among others, for courses from 1-14 days.[155]

In the initial clinical study in 1988, Graham et. al. determined that the incidence of gastric ulcers in patients taking 200*u*g PO QID of misoprostol was significantly less than that of patients taking placebo with concurrent NSAID usage for osteoarthritis.[156] Since that time, numerous other studies and reviews have confirmed the efficacy of the drug.[115,155,157-165] In 1995, the Misoprostol Ulcer Complication Outcomes Safety Assessment (MUCOSA) study performed a

six month, randomized, double-blind, placebo-controlled study of 8,843 patients receiving any of 10 NSAIDs for osteoarthritis.[159] They determined that the overall rate of ulcer complications associated with NSAID use was 40% lower inpatients receiving misoprostol 200 *u*g PO QID, when compared to patients taking placebo.

Evaluation of misoprostol in veterinary medicine

A clinical study of misoprostol for prevention of aspirin-induced gastric injury was performed by Murtaugh et. al. in 1995.[112] A randomized, double-blind study of 18 client-owned dogs that received either aspirin 25 mg/kg PO TID, or aspirin 25 mg/kg PO TID and misoprostol 100 *u*g/dog TID (mean 3.1 *u*g/kg). Endoscopy was performed on days 0 and 14. Lesions were recorded and scored by two observers unaware of the treatment group. At 14 days, dogs in the misoprostol group had significantly lower total lesion scores than dogs in the aspirin only group. Three aspirin-only dogs had to be removed from the study, while all of the misoprostol dogs completed the 14-day course. In addition, dogs in the misoprostol group had significantly less vomiting than dogs in the aspirin group, implying reduction in clinical signs of NSAID toxicity. Diarrhea was noted in dogs from both the aspirin-only group (20/88 days) and the aspirin with misoprostol group (23/125 days) throughout the study. No significant difference in days of diarrhea between groups was detected.

In1995, Johnston et. al. evaluated the efficacy of misoprostol in preventing aspirin-induced injury in dogs.[113] Three groups of 6 adult dogs received 35 mg/kg of aspirin TID, 3 *u*g/kg misoprostol TID or both drugs together for a total of 30 days. Upper GI endoscopy was performed on days 0,5,14 and 30. Five regions of the gastrointestinal tract were scored using a weighted lesion scale, with the lowest value assigned to sub-mucosal hemorrhages and the highest to perforating ulcers. Significant differences in scores were identified between all groups on days 5, 14, and 30, with the most severe lesions in the dogs who received aspirin alone, and the least severe lesions in the misoprostol only group. The study demonstrated that misoprostol 3 *u*g/kg TID was effective in preventing endoscopically detectable gastroduodenal lesions in dogs. There was no

difference in incidence of vomiting between those dogs receiving aspirin alone and those receiving aspirin and misoprostol[113], in contrast to the previous clinical study by Murtaugh et. al., in which administration of misoprostol seemed to result in less vomiting .[112] This difference was attributed to small study size or possibly more significant clinical signs in dogs receiving 35 mg/kg of aspirin versus those receiving 25 mg/kg.[113]

In 1996, Bowersox et. al.[166] studied the effect of higher doses of misoprostol in the prevention of aspirin-induced gastric injury. Twenty random source dogs were divided into four groups which received the following: Group I, no treatment; Group II 35 mg/kg plain aspirin PO TID for 10 days; Group III, 35 mg/kg plain aspirin PO TID and misoprostol 15 *u*g/kg PO TID for five days, then 7.5 *u*g/kg PO TID for five days; and Group IV, misoprostol 15 *u*g/kg PO TID for five days, then 7.5 *u*g/kg PO TID for five days. Upper GI endoscopy was performed at the beginning and end of the treatment period. Each dog was euthanized at the end of the study and histopathology of the gastrointestinal tract evaluated. Four of ten dogs receiving misoprostol at 15 *u*g/kg TID developed diarrhea, which resolved in three dogs without any treatment or adjustment of the dose, and in one dog when the dosage of misoprostol was reduced by half. Dogs in Group II had significantly higher lesion scores when compared to dogs in the other groups. Histopathology did not demonstrate any abnormalities despite the presence of gross lesions in any group except Group II.

In 1997, Johnston et. al. [167]evaluated the efficacy of misoprostol 3 *u*g/kg PO TID in preventing the development of gastroduodenal lesions associated with concurrent administration of piroxicam 0.3 mg/kg PO QD. Twenty-four random source dogs were divided into four groups that received either placebo, misoprostol 3 ug/kg PO TID, piroxicam 0.3 mg/kg PO QD or both misoprostol 3 ug/kg PO TID and piroxicam 0.3 mg/kg PO QD. All dogs received medications for 28 days and gastroduodenoscopy was performed on days 0, 5,14, and 28. Lesions were scored as previously described and median total lesion scores between groups on each day was compared. No statistical difference between

median total gastric lesion scores was detected in any group at any time period, suggesting that piroxicam may not cause significant gastroduodenal lesions in healthy dogs.

Dosage of misoprostol

Due to the rapid metabolism of misoprostol into its unstable intermediates, pharmocologic profiling of the drug has been a cumbersome and largely experimental effort. As a result, dosing of this drug in humans and dogs has been based on the results of a series of clinical trials with arbitrarily selected dosages. The current dose (3 *u*g/kg PO TID) recommended for dogs has been demonstrated to be effective in the study by Johnston et. al.; the TID dosing was selected based on the most recent recommendations in human medicine. Porro et. al. [155] evaluated the efficacy of misoprostol at the standard human dose (200 *u*g) administered TID and BID compared to ranitidine for prevention ofgastric ulceration in patients receiving naproxen for arthritis. All treatments were administered for two weeks and upper GI endoscopy performed at the start and completion of the study. Results for misoprostol administered BID were statistically indistinguishable from those of misoprostol administered TID, both of which were significantly better at preventing ulcers in these patients compared to ranitidine.

Santillan et. al.[168] evaluated the efficacy of misoprostol 50 *u*g (1/4 of the standard human dose) PO BID in preventing gastric injury associated with diclofenac administration in healthy volunteers. Subjects received either diclofenac or diclofenac with misoprostol twice daily for 2 weeks. Upper GI endoscopy was performed on day 14 and gastric lesions scored. Subjects receiving low dose misoprostol had significantly lower lesion scores than the control subjects.

Interactions between NSAIDs and misoprostol

It has been postulated that misoprostol may decrease the absorption of NSAIDS by decreasing the acidity of gastric fluid, which could explain some of the GI protective effect. Several studies in human medicine have not demonstrated

any clinically relevant pharmacokinetic interactions between misoprostol and concurrently administered NSAIDs including aspirin, diclofenac, ibuprofen, indomethacin and piroxicam, except at very high serum salicylate concentrations.[169-171] In one study, no reduction in overall plasma indomethacin concentrations was observed in human subjects receiving concurrent misoprostol; in fact, misoprostol enhanced the steady state maximum concentration of the drug.[172] The mechanism for this enhancement was not defined, but might be related to solubilization of the drug by increased bicarbonate secretion. It is uncertain if this effect is present for other weakly acidic compounds. The effect of misoprostol on the pharmacokinetics of NSAIDs has not been evaluated in veterinary medicine.

Recent in vitro studies have demonstrated that misoprostol augments the anti-inflammatory effects of the NSAIDS piroxicam, indomethacin, and aspirin by suppressing neutrophil superoxide radical formation, as well as neutrophil degranulation and aggregation in systems dependent on cAMP based signal transduction across the plasma membrane.[21] This effect is not present in systems that stimulate neutrophil function and bypass the cAMP signal transduction.[21] This suggests that misoprostol requires a transmembrane signal be transduced which induces a rise in cAMP.[21] This is supported by data that suggests that in vitro, misoprostol reduced the concentration of thromboxane B2 in synovial fluid compared to patients on NSAIDS alone, suggesting an anti-inflammatory effect.[173] Although it is uncertain if the synergistic anti-inflammatory effect of misoprostol and NSAIDs is beneficial in osteoarthritis, it does not appear to reduce the beneficial anti-inflammatory effects of NSAID administration.

Misoprostol: side effects

Misoprostol has had few significant adverse effects in numerous experimental studies . Several studies were done in rats to evaluate the diarrheic potential of misoprostol. Compared to PGE1, misoprostol and its isomers showed less diarrheic potential.[119] Although misoprostol caused transient hypotension in

anesthesized rabbits when administered at 2-4 *u*g/kg IV, conscious dogs showed no change in ECG, heart rate or blood pressure when intragastric doses of 30 *u*g/kg were given.[119] Misoprostol does not effect platelet aggregation, and does not demonstrate estrogenic, progestogenic or adrogenic agonism or antagonism.[119] In doses greater then 30x the antisecretory dose in dogs, no effects were noted on renal, cardiovascular or endocrine systems.[119] No effect on drug metabolizing enzymes was detected in experimental studies in rats.[121] Preclinical studies regarding acute and chronic toxicity of misoprostol in rats and dogs indicate a safety margin of 500-1000 fold between therapeutic doses in humans and lethal doses in animals.[174] Mutagenicity studies were negative and misoprostol was not teratogenic in mice at doses up to 10,000 *u*g/kg, or in rabbits to 1000 *u*g/kg body weight.[174]

Clinical experience with misoprostol in human medicine has shown it to be a safe drug. Of 2,272 patients who participated in several large, multicenter studies, 830 experienced some adverse effects.[175] The most commonly reported side effect was diarrhea. Diarrhea appeared to be dose related, affecting 13.1% of patients receiving 200 *u*g QID, and 9.5%, 4% and 3.8% of patients receiving 100 *u*g, 50*u*g and placebo respectively. Diarrhea was generally mild and self -limiting, with only 4 of 2,272 patients leaving the study for that reason. Other side effects reported include nausea, headaches, vomiting, abdominal pain, malaise and rash. No deaths were attributable to misoprostol in that study. In one recent study evaluating the effectiveness of low dose misoprostol (50 ug BID), diarrhea was observed in only 5% of patients; abdominal pain (11%) and nausea (5%) were the other most common side effects.[168] Experimental data in dogs suggests that stimulation of intestinal fluid secretion as well as inhibition of intestinal motility contribute to the diarrheagenic effect of misoprostol.[176]

Several studies have been performed to evaluate the cost-benefit of misoprostol prophylaxis in human patients receiving NSAIDs.[177 178] In the general population, misoprostol has been associated with increased cost, which outweighs the benefits to quality of life. The use of misoprostol can be associated

with some adverse effects; misoprostol is expensive, may cause diarrhea in some patients, and may not relieve dyspepsic symptoms.[32] A few patients taking misoprostol in one study of patient preferences regarding NSAID prophylaxis reported decreased quality of life while taking misoprostol.[32] This is in contrast to other studies where the benefits of misoprostol therapy to quality of life are better defined.[159] In populations at high risk for developing NSAID-related complications, such as the elderly, those with a history of peptic ulcers, and cigarette smokers[32], prophylaxis with misoprostol has been associated with a more favorable cost-benefit analysis.

Materials and Methods

Twenty-eight random source dogs (14 males, 14 females) were acclimated for a 4-week observation period. All dogs received sulfadimethoxine (Albon, Roche, Nutley, NJ; 50 mg/kg PO once then 25 mg/kg PO D for 14 days), metronidazole (Zenith Goldline, Miami, FL; 50 mg/kg PO QD for 5 days) , and either ivermectin (Ivomec 1%, MSD-Ag Vet, Rahway, NJ; 200 *u*g/kg PO once) or fenbendazole (Panacur, 50 mg/kg PO QD for 3 days) at least 2 weeks prior to the start of the study. Fenbendazole was administered to seven dogs who had a merle coat color suggesting collie genetic material, because collies are sensitive to the neurologic side effects of ivermectin. Each dog was entered into the study on the basis of a normal packed cell volume (reference range 37-55%), total protein (reference range 5.5-7.9 g/dl), blood urea nitrogen dipstick (Azostick, Bayer Corp, Elkhart, IN; reference range 0-25 mg/dl), one negative zinc sulfate fecal flotation, and the lack of erosions or ulceration of the gastric mucosa during initial endoscopic screening evaluation. This study was approved by the Animal Care Committee, Virginia Polytechnic Institute and State University.

Gastroscopy (Fujinon EG7-HRZ Superimage Video Endoscope, or Fujinon EG7-FPZ Video Endoscope, Fijinon Inc, Wayne, NJ, or both) was performed on all dogs 9 days prior to onset of drug administration to determine the presence or absence of *Helicobacter spp*. and to evaluate the appearance of the gastric

mucosa. A gastric mucosal biopsy sample was obtained from the angularis incisura of each dog and a rapid urease test (CLO test, Tri-Med Specialties, Lenexa, KS) was performed on each sample. The gastric mucosal samples were imbedded into the CLO test gel and the media was kept at room temperature for 24 hours. A CLO test was considered positive if the media color changed to red or magenta within 24 hours. A positive result indicated the presence of urease production by *Helicobacter spp.* An additional mucosal biopsy was obtained from one dog, which had a 0.5 cm, non-pigmented, smooth, sessile, polypoid lesion in the pyloric antrum. Histopathology revealed a hyperplastic mucosal polyp, which was considered unlikely to interfere with the experiment, and the dog was kept in the study.

Six dogs were randomly assigned to each of 4 treatment groups, which were stratified so each treatment group contained a similar number of dogs with and without a positive CLO test. Four dogs were excluded from the initial study population because their temperment would not allow administration of oral medication. The dogs received buffered aspirin (Geneva Pharmaceuticals, Inc., Broomfield, CO), misoprostol (Cytotec; G.D. Searle & Co, Chicago, IL), or both as follows: Group I, 25 mg/kg buffered aspirin and methylcellulose placebo PO TID; Group II, buffered aspirin 25 mg/kg PO TID and misoprostol 3 *u*g/kg PO TID; Group III, buffered aspirin 25 mg/kg PO TID, misoprostol 3 *u*g/kg PO BID and methylcellulose placebo PO QD; and Group IV, buffered aspirin 25 mg/kg PO TID, misoprostol 3 *u*g/kg PO QD and methylcellulose placebo PO BID. Misoprostol doses were calculated using five- pound weight increments; capsules were formulated using methylcellulose as an inert base material. Aspirin tablets (325 mg) were broken manually into quarters; each dog received the lowest dose that resulted in a minimum dose of 25 mg/kg. Each dog was initially assigned a dose based on their weight at the onset of the study. Dogs were weighed weekly and doses of aspirin and/or misoprostol were modified if any dog changed weight categories during the study. All treatments were started 9 days after initial endoscopy and continued for 28 consecutive days.

Gastroscopy was performed on all dogs on days –9, 5, 14 and 28 of the study. Food was withheld for 16 hours prior to general anesthesia. Dogs were premedicated with 0.1 mg/kg acepromazine and 0.05 mg/kg atropine sulfate IM. An intravenous catheter was placed and anesthesia was induced with sodium thiopental to effect (approximately 10 mg/kg). All dogs were intubated and maintained on halothane administered via endotracheal tube. Gastroscopy was then performed by an experienced endoscopist (DMW). Endoscopic images were recorded (Sony Promavica Still Video Recorder, Model MVR5300, Sony Corporation, Japan).

The stomach was divided into four regions for the purpose of systematic endoscopic evaluation and scoring as previously described[113]: (1) pylorus and pyloric antrum, (2) angularis incisura, extending along the lesser curvature, (3) gastric body, extending from the cardia along the greater curvature to the pyloric antrum, and (4) the cardia, extending from the greater curvature to the portion of lesser curvature not evaluated with angularis incisura. All dogs were placed in left lateral recumbency. The endoscope was passed into the stomach, which was insufflated to ensure complete visualization of the gastric mucosa. If mucus, hair or other debris was present, water was infused through the endoscope to clear the material and allow adequate visualization of the mucosa. The endoscope was passed into the gastric body initially, then advanced into the antrum where the pylorus was visualized. The endoscope was then retroflexed and the cardia and lesser curvature were scored. Each region of the stomach was scored systematically to reduce the risk of iatrogenic trauma from passage of the endoscope.

Each gastric region was assessed and scored separately on a scale of 1 to 11 as previously described[110,113](Table 1). A submucosal hemorrhage was defined as a petechia or ecchymosis with intact mucosa (Figure 1). Erosion was defined as a superficial defect in the mucosa (Figure 2). Ulceration was defined as a defect in the mucosa associated with observable width, depth and a raised margin (Figure 3). Each region was assigned a score based on the most severe lesion(s) present. If lesions were noted near the border of two contiguous regions,

the lesion was assigned to the most appropriate region and care was taken to not score the lesion twice when evaluating the neighboring region. All scoring was performed by two individuals (ML, SJ) who were unaware of the treatment group. The scores for each region were summed and a total gastric lesion score was assigned to each dog for each day of endoscopy (day –9, 5, 14 and 28). Median scores were calculated for each group on each day of endoscopy.

Serum samples were obtained from all dogs on day 22 for measurement of serum salicylate concentrations. Blood samples were collected 8 hours after oral administration of aspirin and serum was immediately separated. Serum samples were kept at room temperature and delivered to a laboratory (Montgomery Regional Hospital, Blacksburg, VA) for salicylate quantitation using standard fluoroscopic technique (ACA-IV Instrument Manual; E.I. DuPont de Nemours & Company, Inc, Wilmington, DE). All assays were performed within 2 hours of sample acquisition.

All dogs were observed at least three times each day and anorexia and any episode of vomiting and/or diarrhea were recorded. Stool quality was graded on a previously described scale of 1 to 5[110], with a score of 5 assigned to normal, cylindrical stool and a score of 1 assigned to watery stool with no form. A score of 3 was assigned to stool in which greater than two-thirds of the feces were soft, and the stool was firm enough to remain in a pile, but too soft to retain a cylindrical shape. Diarrhea was considered to be present in stool with a score of 3 or less. Anorexia was defined as failure to consume all food offered that day. Dogs were assigned a positive or negative daily value based on the presence or absence of anorexia, or any episode of vomiting or diarrhea each day.

The effect of treatment at each time period and the effects within a treatment group among time periods was determined using a Kruskal-Wallis Rank Sum test.[179] The total lesion scores between each endoscopically evaluated region of the stomach across the treatment periods were evaluated using a Tukey's Honestly Significant Difference Test.[179] The mean serum salicylate concentration, mean dose of aspirin (mg/kg) and mean dose of misoprostol (*u*g/mg) between groups were compared using a Tukey's Honestly Significant

Difference Test.[179] The number of dog-days of anorexia, vomiting and diarrhea were totaled for each group. The number of dog-days of vomiting and dog-days of diarrhea were compared using a Tukey's Honestly Significant Difference Test.[179] The number of days of anorexia in each group was not evaluated statistically as no days of anorexia were noted in any group. $P \leq 0.05$ was considered significant for all statistical tests.

Results

Nine dogs tested positive for *Helicobacter spp*. based on the results of the CLO test. Two positive dogs were assigned to each group, except for Group III, which randomly received 3 positive dogs. There was no significant difference in median total gastric lesion scores between any groups prior to the start of the study on day –9 (Table 2). All dogs that were included in the study received a gastric lesion score of 1(no lesions) in all four areas of the stomach, except for one dog in Group II, which received a score of 2 (1 submucosal hemorrhage) in the cardia and a score of 3 (2-5 submucosal hemorrhages) in the pyloric antrum. This dog was kept in the study in order to maintain an equal number of dogs in each group. The hemorrhages noted were very small, and considered unlikely to represent significant gastric mucosal disease.

Figure 4 demonstrates the median total gastric lesion scores for each group for each day of endoscopy. Significant differences in median total gastric lesion scores between groups were identified at 5, 14, and 28 days (Table 2). No differences in median total gastric lesion score were identified between Group I (control; 16, 19.5, 21) and Group IV (misoprostol QD; 19, 20.5, 23) on days 5, 14 and 28 respectively. Median total gastric lesion score was significantly lower ($p \leq 0.05$) in Groups II (misoprostol TID; 7) and III (misoprostol BID; 6) compared to Groups I (control;16) and IV (misoprostol QD; 19) on day 5. On day 14, median total gastric lesion score of Group III (misoprostol BID; 16) was significantly lower ($p \leq 0.05$) than that of Group IV (misoprostol QD; 20.5), but not Group I (control; 19.5). No significant difference was identified between Group II (misoprostol TID;

16.5) and Groups I (control; 19.5), III (misoprostol BID; 16) and IV (misoprostol QD; 20.5) on day 14. Group III (misoprostol BID; 10) had a significantly lower median total gastric lesion score ($p \leq 0.05$) compared to Groups I (control;21), II (misoprostol TID;18.5) and IV (misoprostol QD;23) on day 28. No significant difference was identified between Group II (misoprostol TID; 18.5) and Groups I (control; 21), Group III (misoprostol BID; 10) and IV (misoprostol QD; 23) on day 28.

There was a significant increase in median total gastric lesion score in Group I (control) from day –9 to day 5, but not from day 5 to day 14, or day 14 to day 28 (Figure 4). Group II (misoprostol TID) demonstrated a significant increase in median total gastric score from day 5 to day 14, but not between day –9 to day 5, or day 14 to day 28. Median total lesion scores for Group III (misoprostol BID) did not rise significantly from day –9 to day 5. A significant increase in median total gastric lesion score was observed for Group III (misoprostol BID) from day 5 to day 14, and a significant decrease in median total gastric lesion score was noted from day 14 to day 28. There was a significant increase in median total gastric lesion score in Group IV(misoprostol QD) from day –9 to day 5, but not from day 5 to day 14, or from day 14 to day 28.

When total lesion scores for each region of the stomach were averaged across all treatment days, the mean total gastric lesion scores of the cardia (3.75) and greater curvature (3.61) were significantly greater than mean total lesion scores of the angularis incisura (3.03) and pyloric antrum (2.49). The mean total lesion score of the pyloric antrum was significantly less than the mean total lesion scores of the cardia, greater curvature and angularis incisura. Individual comparisons of regional lesion scores on each endoscopy day and between groups could not be performed because the number of observations was too small for statistical analysis.

No significant difference was found between the mean misoprostol doses of Groups II, III, and IV (3.02 *u*g/kg, 3.12 *u*g/kg and 3.01 *u*g/kg, respectively). No significant difference was found between mean aspirin doses of Groups I, II, III, and IV (26.63 mg/kg, 26.79 mg/kg, 27.01 mg/kg, and 26.25 mg/kg, respectively).

The mean serum salicylate concentration of Group II (16.95 mg/dl) was significantly greater ($p \leq 0.05$) than the mean serum salicylate concentrations of Group I, Group III and Group IV (12.82 mg/dl, 12.27 mg/dl, and 13.33 mg/dl, respectively).

Vomiting was observed in Groups II, III and IV, but not in Group I during the study period. There was 1 dog-day of vomiting in Group II, 3 dog-days of vomiting in Group III and 13 dog-days of vomiting in Group IV. No significant difference between mean dog-days of vomiting was noted between any group. Vomiting was self-limiting in all dogs. No decrease in appetite was observed in any dog associated with vomiting. Although one dog in Group IV experienced 7 dog-days of vomiting, most dogs who vomited experienced 1 to 3 dog-days of vomiting intermittently through the study period. No hematemesis was noted .

Nineteen dog-days of diarrhea were observed in Group I, 20 dog-days in Group II, 50 dog-days in Group III and 11 dog-days in Group IV. No significant difference of mean dog-days of diarrhea between any groups was detected. Diarrhea was observed in 10 dogs (range 2 – 23 dog-days/dog). Of these, 7 dogs experienced 7 or more dog-days of diarrhea during the study. Although most dogs had several episodes of diarrhea a day, no evidence of dehydration, or anorexia was noted in any dog with diarrhea. All dogs that had diarrhea during the study had a negative zinc sulfate fecal flotation during the last 7 days of the study. All dogs experiencing diarrhea had a fecal clostridial enterotoxin reverse latex agglutination test (RPLA Kit, Microbio, Denver, CO) during the last 7 days of the study. Two dogs experiencing diarrhea were positive for clostridial enterotoxin. Both of these dogs belonged to Group I. Ten dogs with diarrhea at the end of the study were observed for an additional 6 days. Diarrhea resolved in 9 dogs within 6 days of completing the study. Of these, 2 had been receiving aspirin and placebo (Group I), 2 had been receiving aspirin and misoprostol (Group II), and 5 had been receiving aspirin, misoprostol and placebo (4 dogs in Group III; 1 dog in Group IV). One dog from Group I was observed for an additional 5 days and resolution of the diarrhea did not occur. This dog was positive for fecal clostridial enterotoxin.

Discussion

The goal of this study was to evaluate the efficacy of misoprostol 3 *u*g/kg PO BID or QD compared to misoprostol 3 *u*g/kg PO TID in preventing the development of endoscopically detectable gastric lesions in dogs receiving aspirin 25 mg/kg PO TID. Misoprostol is a synthetic prostaglandin E1 analogue that has been proven to prevent the development of gastric mucosal lesions in humans[41,115,155-165,168] and dogs [112,113,166] during NSAID administration. Proposed mechanisms of action for misoprostol include stimulation of mucus and bicarbonate secretion[137-139,145-149], alteration of mucosal blood flow[119,130,132,147,150], and enhancement of epithelial cell turnover.[140-144] Ideally, selection of a dose and dosing interval for a medication should be made on the basis of pharmacokinetic and pharmacodynamic data. However, as misoprostol is rapidly de-esterified to its acid counterpart after oral administration, and the active metabolite of the drug is uncertain[119], dosing in both human and veterinary medicine has been extrapolated from a series of clinical studies. Previous studies have demonstrated that misoprostol at a dosage of 3 *u*g/kg PO TID is effective in protecting the canine gastric mucosa against concurrent administration of aspirin at dosages of 25[112] and 35[113] mg/kg PO TID. To the authors' knowledge, the efficacy of doses of misoprostol lower than 3 *u*g/kg or less frequent administration than TID has not been previously evaluated in dogs.

Reduction of the total daily dosage of misoprostol in this study was accomplished by reducing the frequency of administration of the clinically recommended TID dose (3 *u*g/kg) to BID and QD, rather than evaluating a lower dose given TID. This study design was selected for several reasons. In humans, TID administration of 200 *u*g has been the standard, as it has been demonstrated to be effective and associated with fewer side effects than QID dosing.[160] However, recent endoscopic studies have demonstrated the efficacy of 200 *u*g of misoprostol administered BID to be equal to that of 200 *u*g administered TID or QID.[155,157] In addition, side effects such as abdominal pain, flatulence, loose or

watery stools, dyspepsia and nausea, were less in those patients receiving BID administration then those receiving QID dosing.[157] Several randomized studies in human medicine have demonstrated that decreased frequency of administration of medications is associated with better compliance and more consistent dosing.[180,181] Presumed advantages of BID or QD dosing of misoprostol in dogs receiving concurrent NSAIDs could include reduction of expense, decreased incidence of side effects, and improved owner compliance with administration of medications.

In this study, median total gastric lesion scores for dogs receiving aspirin with misoprostol 3 *u*g/kg PO BID were significantly lower than those of the aspirin-only group on 2 of 3 days evaluated (days 5 and 28). Although a trend towards lower scores in the misoprostol BID group was noted compared to the aspirin-only group on day 14, no statistical difference was observed. These data demonstrate that BID administration of misoprostol was effective in reducing the gastric lesions associated with aspirin therapy. In this model, misoprostol 3 *u*g/kg PO BID was also as effective as misoprostol 3 *u*g/kg PO TID in preventing the development of gastric lesions with concurrent administration of aspirin 25 mg/kg PO TID. Median total gastric lesion scores for dogs receiving aspirin with misoprostol 3 *u*g/kg PO QD were statistically indistinguishable from those of dogs receiving only aspirin, suggesting that 3 *u*g/kg of misoprostol given once daily is not effective for preventing aspirin-induced gastric injury in the dog.

In this study, misoprostol 3 *u*g/kg PO TID was less effective in preventing the development of gastric lesions than in previous reports.[112,113] Misoprostol administered at 3 *u*g/kg PO TID resulted in statistically lower median total gastric lesion scores compared to the aspirin-only control on day 5. However, no statistical difference was observed between the scores of the misoprostol TID group when compared to the aspirin-only group on days 14 and 28, although a trend towards lower scores in the misoprostol TID group was present. There are several possible explanations for this deviation from expected results, including small sample size, individual variation in sensitivity to aspirin effects, and higher

serum salicylate concentrations in the misoprostol TID group compared to the other groups.

It is possible that the small sample size (6 dogs per treatment group) combined with individual variation in sensitivity to NSAIDs made determining statistical significance between some groups difficult. Several studies in human medicine have suggested that individual sensitivity to NSAIDs affects the development of gastrointestinal complications, although the exact mechanism is undetermined.[182] Individual variation in absorption of aspirin may be a factor in this individual sensitivity.[182] In previous studies evaluating the effects of NSAID administration on the canine gastric mucosa, variation in the severity of lesions between individual dogs has been a consistent finding.[106,108,110] This is supported in this study by the broad range of total gastric lesion scores. In addition, in previous studies of the effects of NSAIDs on the gastrointestinal tract, development of lesions has been demonstrated by endoscopy in dogs receiving only placebo.[108] The authors speculated that stress of laboratory confinement may result in mild lesions in dogs, or that this finding may represent variation among individual dogs.

Despite this individual variation, endoscopic scoring of gastric mucosal injury remains an effective method of evaluating the effect of NSAIDs on the gastric mucosa. The endoscopic appearance of the canine gastric mucosa has been shown to correlate well to histopathologic evaluation of the tissue.[183] Endoscopy is a more sensitive indicator of NSAID-induced gastric injury than clinical signs. Twenty-five percent of NSAID-induced gastric ulcers in people may be asymptomatic, and as many as 50% of patients may not show classical clinical signs.[25,32,35] In at least one study in humans receiving aspirin and misoprostol, no correlation could be made between clinical signs of gastrointestinal discomfort and endoscopic lesion scores.[165] Several canine studies have demonstrated significant mucosal injury associated with administration of aspirin without detectable clinical signs.[108,109] Most canine[108-110,112,113,166] and human

studies[64,86,116,155,157] documenting the effects of NSAIDs on the stomach have used a variation of the endoscopic scoring scale used here.

When samples for trough serum salicylate concentrations obtained on day 22 of the study were evaluated, the mean total serum salicylate concentration for Group II, which received misoprostol TID, was significantly higher than that of the other groups. It is possible that the higher serum salicylate concentrations in this group resulted in more severe gastric lesions. This could explain the lack of significant protection demonstrated by the misoprostol TID group on days 14 and 28. When the amount of aspirin administered to each dog was calculated for each group, there was no statistical difference between mean serum salicylate dose for any group. Therefore, factors other than dosage must have influenced serum concentration.

Marked variability of serum salicylate concentrations has been demonstrated experimentally in dogs receiving aspirin.[30] Drug formulation, gastric emptying time, stomach content, pH of gastric fluid and concurrent drug administration are all thought to effect absorption of aspirin in dogs.[24] Although the dogs of this study were fed the same diet in a controlled environment, all dogs were allowed to eat ad libitum throughout the day, which could have resulted in variability in gastric contents at the time medications were administered. The formulation of aspirin we used, buffered aspirin, has been shown to produce relatively consistent serum salicylate concentrations in the dog[29], so aspirin formulation was not likely a factor in the variability of serum salicylate concentration. Serum salicylate concentrations for all dogs were within the recommended canine therapeutic range (5 - 30 mg/dL).[30]

Aspirin has been shown to compete with misoprostol for protein-binding sites in humans at very high serum concentrations of salicylate.[123] Since this effect has not been evaluated in dogs, it is difficult to know at what serum salicylate concentration these two drugs might interact in dogs. Since evaluating serum concentration of misoprostol is not technically feasible, we can not ascertain whether or not the dogs receiving misoprostol TID had higher serum

levels of misoprostol compared to other groups, although we might anticipate that they would, due to the higher total daily dose in this group. It is possible that the higher dose of misoprostol might have altered the transport of salicylate, resulting in higher free serum salicylate concentrations and more severe gastric lesions. Alternatively, elevated serum salicylate concentrations could have affected the plasma transport of misoprostol, and reduced its effectiveness compared to other groups.

In this study, we used an aspirin dose of 25 mg/kg given TID. This dose of aspirin has been shown to cause endoscopically detectable gastric lesions in dogs, ranging from petechiation to linear hemorrhages.[29] In a previous study of similar design[113], misoprostol 3 *u*g/kg TID was proven to reduce endoscopic lesion scores with an aspirin dose of 35 mg/kg TID. It is possible that the higher dose of aspirin resulted in more severe lesions in the aspirin-only group in that study, which could have resulted in greater statistical difference between groups. Therefore the 35 mg/kg TID model may be more effective at generating statistically significant differences. However, misoprostol (3 *u*g/kg TID) significantly reduced the severity of gastric injury associated with concurrent administration of aspirin at an average dose of 25 mg/kg PO TID in a series of clinical patients[112], suggesting the lower aspirin dose could be a useful model. Since the 25 mg/kg dose of aspirin is within the most commonlyclinically recommended range of aspirin doses for dogs (10-25 mg/kg), we chose to use this dose instead of the higher dose, to evaluate the effects of aspirin and misoprostol as they would pertain to clinical patients. In previous studies from this laboratory, some morbidity associated with NSAID-induced gastric lesions was observed in dogs that received 35 mg/kg of aspirin PO TID for several weeks. By using the lower dose (25 mg/kg PO TID), we hoped to reduce the incidence of clinical illness in the study dogs, while still providing an effective model for the study. In addition, the previously mentioned study used plain aspirin, rather than buffered aspirin. Plain aspirin at 25 mg/kg TID has been shown to produce more serious endoscopically-detectable gastric lesions in dogs compared to the same dose of buffered aspirin.[29]

Humans that develop gastric lesions within hours or days of starting NSAID therapy can show resolution of these lesions despite continued administration[184]. This process has been termed gastric adaptation, and although the mechanism is undetermined, it appears to be prostaglandin dependent.[108] In some canine studies on the effects of NSAIDs on the gastric mucosa, spontaneous resolution of gastric lesions has been noted as early as 3 days after initiation of therapy.[105,106] It is unlikely that this effect explains the failure of Group II (misoprostol TID) to demonstrate efficacy on days 14 and 28, as the lesion scores for Group II (misoprostol TID) and Group III (misoprostol BID) rise on day 14, and those of Group II (misoprostol TID) remain high on day 28 (Figure 4). The median total gastric lesion scores for the aspirin group remained elevated throughout the study. Therefore, the failure to demonstrate significant differences in scores between those groups on those days seems to result from failure of the misoprostol treatment to reduce the severity of gastric injury, rather than improvement from gastric adaptation in the control group. It is possible that the lower median total gastric lesion score demonstrated by Group III (misoprostol BID) on day 28 could reflect gastric adaptation, rather than a true effect of treatment. However, this seems unlikely as we would expect this effect to be relatively constant across treatment groups as they all received the same dose of aspirin; Group II should be no more likely to experience this effect than the others. Since the process of gastric adaptation may be prostaglandin-dependent, it is possible that misoprostol, as a prostaglandin analogue, could enhance the process. Since Group II (misoprostol TID) received the highest daily dose of misoprostol, we would expect the effect on gastric adaptation to be greatest in Group II (misoprostol TID), making it less likely to singularly effect Group III (misoprostol BID) on day 28.

In this study, the duodenum was not evaluated or scored using endoscopy. Although NSAIDs have been shown to cause the development of lesions in the duodenum of humans and dogs[25,32,109], previous experimental studies in dogs from this laboratory that have evaluated the duodenum have shown minimal

lesions in that region.[110,113,167] Since duodenal intubation results in considerably more endoscopy time, and was considered unlikely to result in additional relevant information, gastroscopy alone was performed.

In this study, statistically significant differences were found in total gastric lesion score between gastric regions. The pyloric antrum was the area of the stomachwhichwas least affected; followed by the angularis incisura. The highest lesion scores were obtained from the cardia and greater curvature. This is in contrast to human studies[87], veterinary clinical reports[29,109,111] and some endoscopic studies in dogs[112], in which the pyloric antrum appears to be the gastric region most severely affected by NSAIDs. In recent endoscopic studies in dogs evaluating the effects of aspirin, etodolac and carprofen on the gastric mucosa[110,113], no region of the stomach was any more likely than another to develop mucosal lesions.

Administration of misoprostol 3 *u*g/kg PO BID did not result in increased gastrointestinal signs of anorexia, vomiting or diarrhea compared to aspirin alone. Diarrhea is one of the most frequently reported side effects of misoprostol reported in humans.[157,175] Generally diarrhea is mild and self-limiting. In previous canine studies, diarrhea wasnot a major problem when misoprostol was administered at a dose of 3 *u*g/kg TID. However, severe diarrhea was seen in one canine study when doses of 15 *u*g/kg were administered; diarrhea resolved spontaneously in 3 dogs in that study, and resolved in another when the dose of misoprostol was reduced to 7.5 *u*g/kg.[166] Dehydration and weight loss associatedwithdiarrhea were not observed in any dog. Since some dogs in all groups exhibited diarrhea, and since all but one dog had resolution of diarrhea with discontinuation of drugs, we conclude that diarrhea was most likely not associated with misoprostol. Possible causes of diarrhea in this study include aspirin and methycellulose.

Conclusions

Based on this study, misoprostol 3 *u*g/kg PO BID was as effective as misoprostol 3 *u*g/kg PO TID in preventing aspirin-induced gastric mucosal injury in dogs. Administration of misoprostol 3 *u*g/kg PO BID did not result in increased incidence of diarrhea, vomiting, or anorexia when compared to aspirin alone. Advantages of decreased frequency of administration of misoprostol include improved owner compliance, reduced effort of treatment, and reduced cost of therapy with equal efficacy in the prevention of aspirin-induced gastric injury. Further study is needed to confirm the efficacy of misoprostol 3 *u*g/kg BID in preventing aspirin-induced gastric injury in dogs with clinical signs requiring NSAID therapy.

Tables

Table 1. Numerical value assigned to each region of the stomach based on the following criteria[a] as recognized from gastroscopy.

Score	Description
1	Normal
2	1 Submucosal hemorrhage
3	2-5 Submucosal hemorrhages
4	>5 Submucosal hemorrhages
5	1 Erosion
6	2-5 Erosions
7	>5 Erosion
8	1 Ulcer
9	2 Ulcers
10	> 3 Ulcers
11	Perforating Ulcer

[a] Criteria previously published by Reimer et. al.[110]

Table 2. Median Total Gastric Lesion Score for Dogs Receiving Aspirin Alone or with Misoprostol on All Days of Endoscopic Evaluation of the Gastric Mucosa

Group Number[b]	Day –9	Day 5	Day 14	Day 28
I	4	16	19.5	21
II	4	7	16.5	18.5
III	4	6	16	10
IV	4	19	20.5	23

[b]Group I: Buffered aspirin 25 mg/kg PO TID and placebo PO TID
Group II: Buffered aspirin 25 mg/kg PO TID and misoprostol 3 *u*g/kg PO TID
Group III: Buffered aspirin 25 mg/kg PO TID, misoprostol 3 *u*g/kg PO BID, placebo PO QD
Group IV: Buffered aspirin 25 mg/kg PO TID, misoprostol 3 *u*g/kg PO QD, placebo PO BID

Figures

Figure 1. Multiple submucosal hemorrhages on the angularis incisura of a dog. This dog received a lesion score of 4.

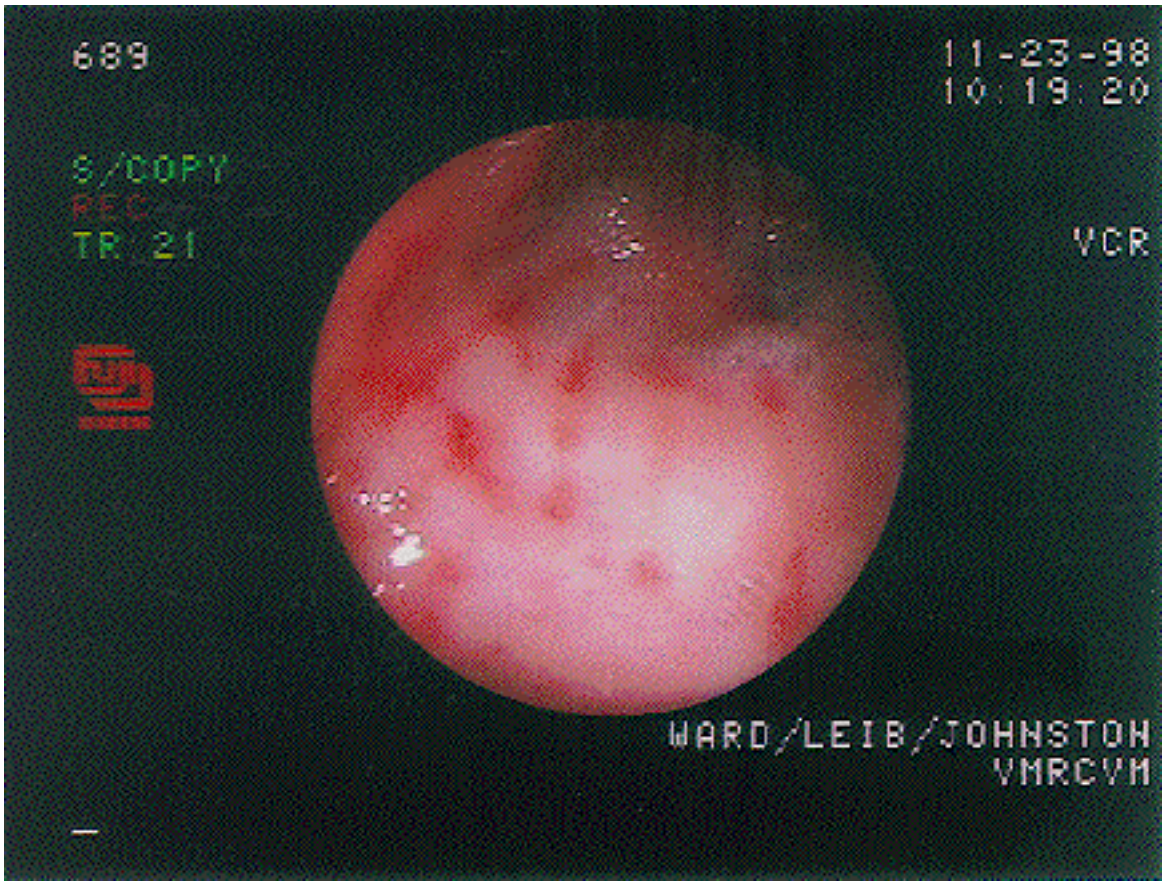


Figure 2. Several linear erosions along the greater curvature of the stomach of a dog. Erosions are characterized by discontinuous epithelium. This dog received a lesion score of 7.

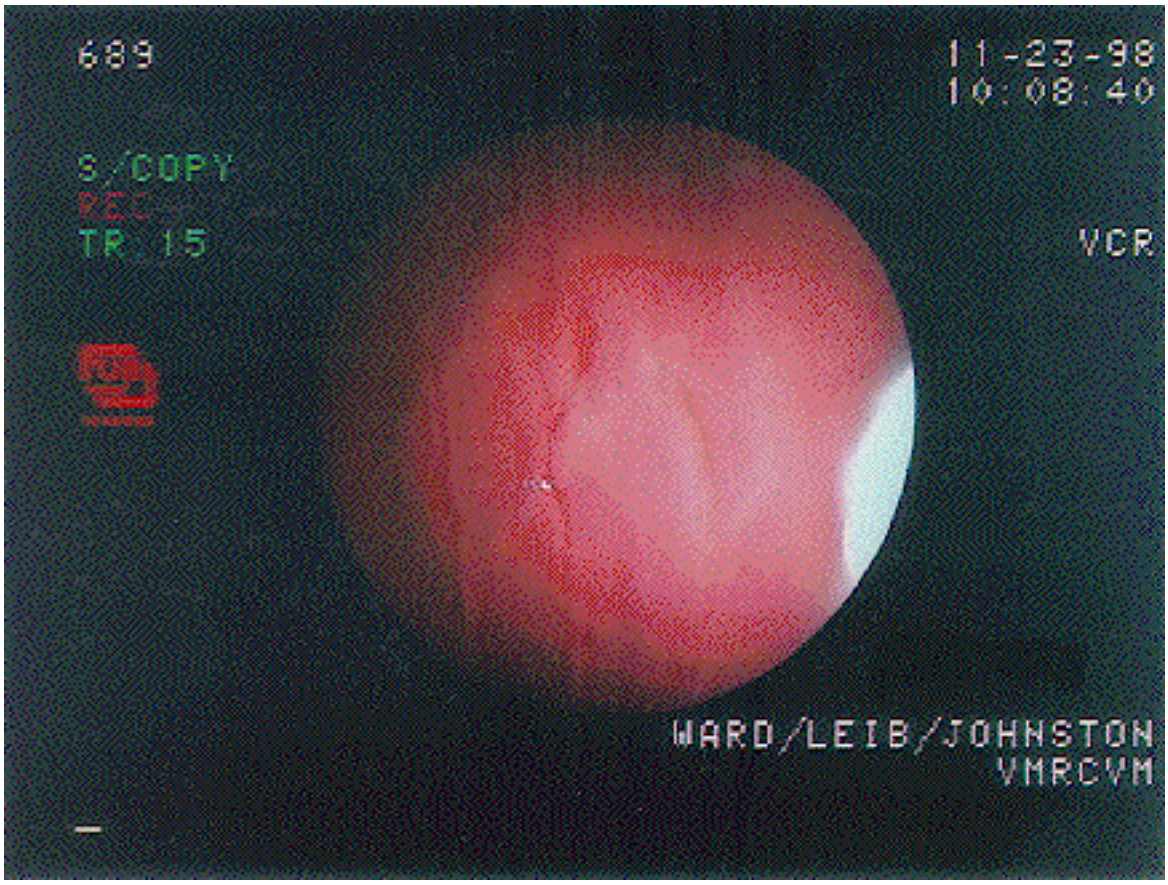


Figure 3. An ulcer in the pyloric antrum of a dog receiving aspirin and misoprostol. Note the central crater and raised margin suggesting severe discontinuation of the epithelium. This dog received a lesion score of 8.

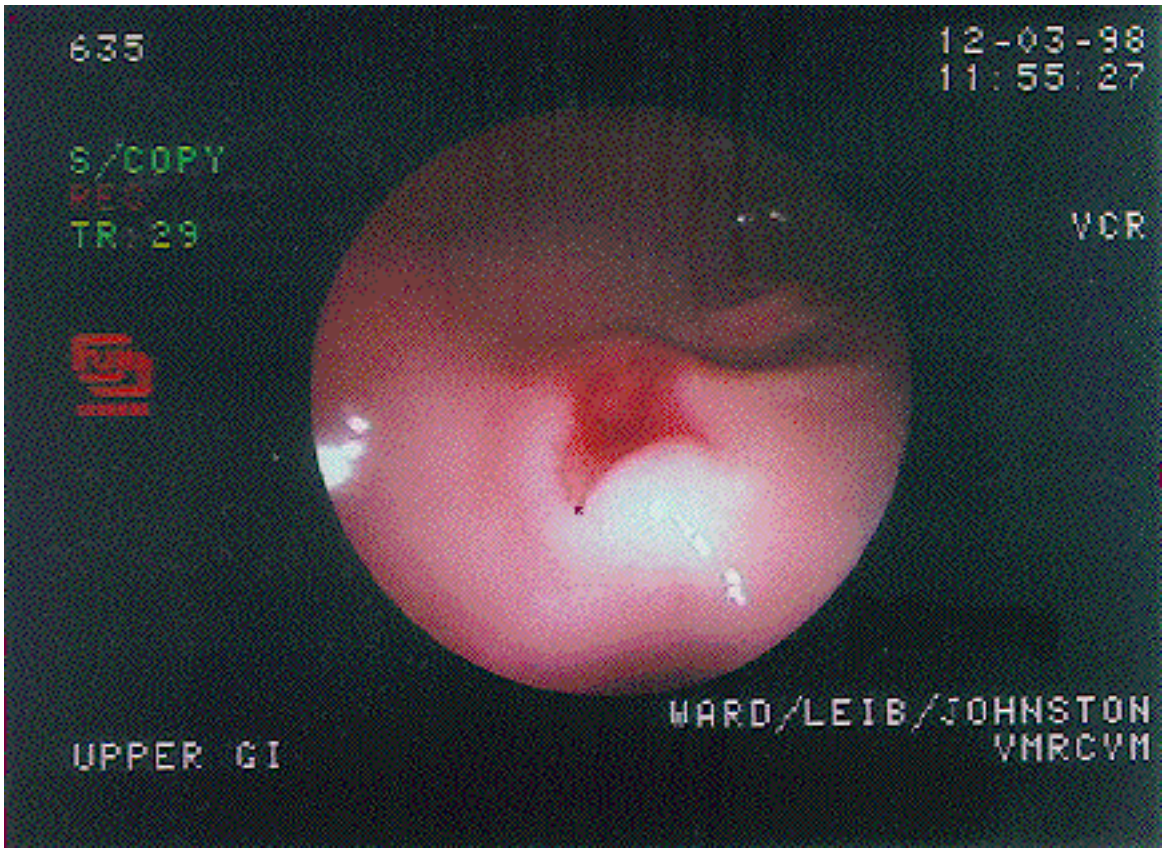
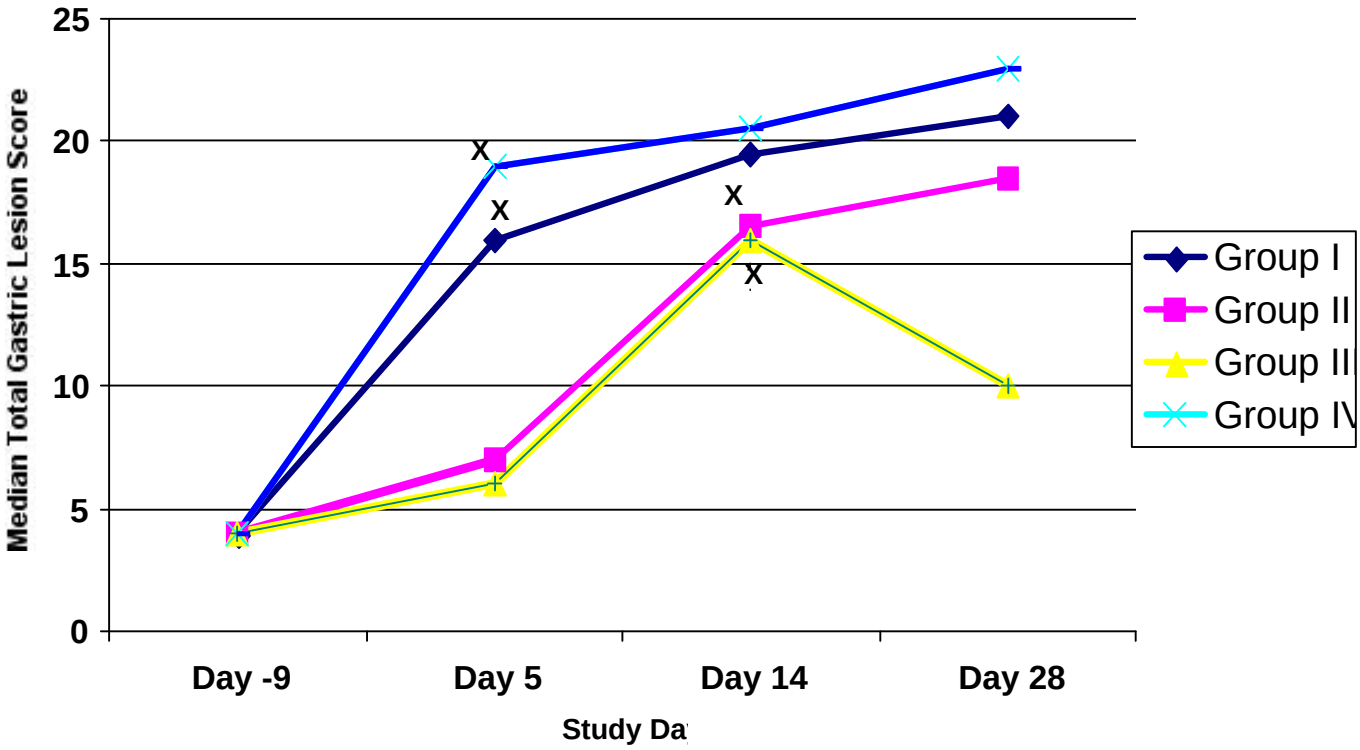


Figure 4. Median Total Gastric Lesion Score for D
Receiving Aspirin Alone or Aspirin and Misopro



On Day 5, median total gastric lesion scores for Groups II and III were significantly lower than that of Groups I and IV.

On Day 14, median total gastric lesion score for Group III was significantly lower than that of Group IV.

On Day 28, median total gastric lesion score for Group III was significantly lower than that of Groups I, II, and IV.

[x] Indicates that significant increase or decrease in median total lesion score was detected from the previous endoscopy score for the group indicated

Appendices

Appendix One: Results of CLOtest for Individual Dogs [a]

Treatment - Group Number	Individual Dog Number	CLO Test Result (+/-)
Control - I	661	+
Control - I	689	+
Control - I	633	-
Control - I	681	-
Control - I	663	-
Control - I	640	-
Misoprostol TID - II	659	+
Misoprostol TID - II	656	+
Misoprostol TID - II	635	-
Misoprostol TID - II	693	-
Misoprostol TID - II	672	-
Misoprostol TID - II	646	-
Misoprostol BID - III	660	+
Misoprostol BID - III	645	+
Misoprostol BID - III	650	+
Misoprostol BID - III	636	-
Misoprostol BID - III	682	-
Misoprostol BID - III	658	-
Misoprostol QD - IV	648	+
Misoprostol QD - IV	664	+
Misoprostol QD - IV	639	-
Misoprostol QD - IV	677	-
Misoprostol QD - IV	568	-
Misoprostol QD - IV	651	-

[a] Clotest, Trimed Specialities, Lenexa, KS

Appendix Two: Results of Endoscopic Scoring of Each Gastric Region and Total Gastric Lesion Scores in Dogs Receiving Aspirin Alone or Aspirin with Misoprostol

A. Regional and Total Gastric Lesion Scores for Individual Study Dogs on Day -9

Treatment - Group Number	Dog Number	Greater Curvature	Pyloric Antrum	Angularis Incisura	Cardia	Total Gastric Lesion Score
Control - I	661	1	1	1	1	4
Control - I	689	1	1	1	1	4
Control - I	633	1	1	1	1	4
Control - I	681	1	1	1	1	4
Control - I	663	1	1	1	1	4
Control - I	640	1	1	1	1	4
Misoprostol TID - II	659	1	1	1	1	4
Misoprostol TID - II	656	1	1	1	1	4
Misoprostol TID - II	635	1	1	1	1	4
Misoprostol TID - II	693	1	3	1	2	7
Misoprostol TID - II	672	1	1	1	1	4
Misoprostol TID - II	646	1	1	1	1	4
Misoprostol BID - III	660	1	1	1	1	4
Misoprostol BID - III	645	1	1	1	1	4
Misoprostol BID - III	650	1	1	1	1	4
Misoprostol BID - III	636	1	1	1	1	4
Misoprostol BID - III	682	1	1	1	1	4
Misoprostol BID - III	658	1	1	1	1	4
Misoprostol QD - IV	648	1	1	1	1	4
Misoprostol QD - IV	664	1	1	1	1	4
Misoprostol QD - IV	639	1	1	1	1	4
Misoprostol QD - IV	677	1	1	1	1	4
Misoprostol QD - IV	568	1	1	1	1	4
Misoprostol QD - IV	651	1	1	1	1	4

B. Regional and Total Gastric Lesion Scores for Individual Study Dogs on Day 5

Treatment - Group Number	Dog Number	Greater Curvature	Pyloric Antrum	Angularis Incisura	Cardia	Total Gastric Lesion Score
Control - I	661	6	2	1	3	12
Control - I	689	7	6	3	6	22
Control - I	633	4	4	5	6	19
Control - I	681	5	4	5	2	16
Control - I	663	1	4	3	3	11
Control - I	640	6	1	3	6	16
Misoprostol TID - II	659	1	1	1	1	4
Misoprostol TID - II	656	3	1	3	4	11
Misoprostol TID - II	635	1	1	1	3	6
Misoprostol TID - II	693	6	6	3	6	21
Misoprostol TID - II	672	1	1	1	1	4
Misoprostol TID - II	646	2	1	2	3	8
Misoprostol BID - III	660	3	1	1	5	10
Misoprostol BID - III	645	2	1	1	1	5
Misoprostol BID - III	650	2	1	3	3	9
Misoprostol BID - III	636	1	1	1	1	4
Misoprostol BID - III	682	4	1	1	1	7
Misoprostol BID - III	658	1	1	1	1	4
Misoprostol QD - IV	648	4	6	3	3	16
Misoprostol QD - IV	664	4	3	6	6	19
Misoprostol QD - IV	639	4	1	3	4	12
Misoprostol QD - IV	677	6	1	6	6	19
Misoprostol QD - IV	568	6	4	4	6	20
Misoprostol QD - IV	651	7	3	6	3	19

C. Regional and Total Gastric Lesion Scores for Individual Study Dogs on Day 14

Treatment - Group Number	Dog Number	Greater Curvature	Pyloric Antrum	Angularis Incisura	Cardia	Total Gastric Lesion Score
Control - I	661	7	6	6	6	25
Control - I	689	7	3	3	6	19
Control - I	633	1	3	6	1	11
Control - I	681	3	4	3	3	13
Control - I	663	6	3	6	6	21
Control - I	640	7	1	6	6	20
Misoprostol TID - II	659	6	3	1	6	16
Misoprostol TID - II	656	6	1	5	6	18
Misoprostol TID - II	635	5	8	6	6	25
Misoprostol TID - II	693	7	1	1	6	15
Misoprostol TID - II	672	3	1	1	2	7
Misoprostol TID - II	646	3	1	7	6	17
Misoprostol BID - III	660	6	1	4	8	19
Misoprostol BID - III	645	1	1	1	5	8
Misoprostol BID - III	650	6	1	3	5	15
Misoprostol BID - III	636	7	6	6	6	25
Misoprostol BID - III	682	1	3	3	3	10
Misoprostol BID - III	658	5	4	1	7	17
Misoprostol QD - IV	648	6	3	5	6	20
Misoprostol QD - IV	664	7	3	3	7	20
Misoprostol QD - IV	639	7	1	7	7	22
Misoprostol QD - IV	677	7	6	7	6	26
Misoprostol QD - IV	568	1	4	6	7	18
Misoprostol QD - IV	651	6	6	6	3	21

D. Regional and Total Gastric Lesion Scores for Individual Study Dogs on Day 28

Treatment - Group Number	Dog Number	Greater Curvature	Pyloric Antrum	Angularis Incisura	Cardia	Total Gastric Lesion Score
Control - I	661	7	3	6	6	22
Control - I	689	7	3	6	7	23
Control - I	633	6	3	5	3	17
Control - I	681	3	2	6	2	13
Control - I	663	7	5	3	6	21
Control - I	640	7	1	6	7	21
Misoprostol TID - II	659	6	5	6	6	23
Misoprostol TID - II	656	2	1	1	4	8
Misoprostol TID - II	635	6	6	1	7	20
Misoprostol TID - II	693	7	1	2	7	17
Misoprostol TID - II	672	1	1	1	1	4
Misoprostol TID - II	646	7	6	6	7	26
Misoprostol BID - III	660	7	1	2	2	12
Misoprostol BID - III	645	1	6	1	3	11
Misoprostol BID - III	650	6	1	6	7	20
Misoprostol BID - III	636	1	2	1	3	7
Misoprostol BID - III	682	1	2	2	3	8
Misoprostol BID - III	658	1	1	1	1	4
Misoprostol QD - IV	648	7	6	6	6	25
Misoprostol QD - IV	664	1	6	7	7	21
Misoprostol QD - IV	639	10	7	7	7	31
Misoprostol QD - IV	677	7	6	6	6	25
Misoprostol QD - IV	568	1	5	7	7	20
Misoprostol QD - IV	651	6	3	2	6	17

Appendix Three: Mean Dose of Misoprostol Administered to Individual Study Dogs Receiving Aspirin Alone or Aspirin with Misoprostol Over the Entire Study Period

Treatment - Group Number	Dog Number	Mean Body Weight (kg)	Amount of Misoprostol per Dose (*u*g)	Mean dose of Misoprostol (*u*g/kg)
Control - I	661	18.8	0	N/A
Control - I	689	19.4	0	N/A
Control - I	633	17.4	0	N/A
Control - I	681	21.5	0	N/A
Control - I	663	14.5	0	N/A
Control - I	640	21.7	0	N/A
Misoprostol TID - II	659	16.9	55	3.26
Misoprostol TID - II	656	24.5	75	3.06
Misoprostol TID - II	635	24.8	75	3.01
Misoprostol TID - II	693	25.1	75	2.99
Misoprostol TID - II	672	19.3	55	2.84
Misoprostol TID - II	646	23.3	68	2.91
Misoprostol BID - III	660	22.7	68	2.99
Misoprostol BID - III	645	15.1	48	3.17
Misoprostol BID - III	650	15.8	48	3.03
Misoprostol BID - III	636	16.7	55	3.29
Misoprostol BID - III	682	17.6	55	3.12
Misoprostol BID - III	658	17.6	55	3.12
Misoprostol QD - IV	648	24.9	75	3.01
Misoprostol QD - IV	664	20.2	61	3.01
Misoprostol QD - IV	639	22.2	68	3.06
Misoprostol QD - IV	677	21.5	61	2.84
Misoprostol QD - IV	568	22.8	68	2.98
Misoprostol QD - IV	651	21.5	68	3.16

Mean Total Misoprostol Dose for Each Group over Entire Study Length

Treatment - Group Number	Mean Misoprostol Dose (*u*g/kg)
Control - I	N/A
Misoprostol TID - II	3.02
Misoprostol BID - III	3.12
Misoprostol QD - IV	3.01

Appendix Four: Mean Dose of Aspirin Administered to Individual Study Dogs Receiving Aspirin Alone or Aspirin with Misoprostol Over the Entire Study Period

Treatment - Group Number	Dog Number	Mean Body Weight (kg)	Amount of Aspirin per Dose (mg)	Mean dose of Aspirin (mg/kg)
Control - I	661	18.8	488	26.0
Control - I	689	19.4	488	25.1
Control - I	633	17.4	488	28.1
Control - I	681	21.5	568	26.4
Control - I	663	14.5	406	27.9
Control - I	640	21.7	568	26.2
Misoprostol TID - II	659	16.9	488	28.9
Misoprostol TID - II	656	24.5	650	26.6
Misoprostol TID - II	635	24.8	650	26.2
Misoprostol TID - II	693	25.1	650	25.9
Misoprostol TID - II	672	19.3	488	25.2
Misoprostol TID - II	646	23.3	650	27.9
Misoprostol BID - III	660	22.7	568	25.0
Misoprostol BID - III	645	15.1	406	26.8
Misoprostol BID - III	650	15.8	406	25.6
Misoprostol BID - III	636	16.7	488	29.2
Misoprostol BID - III	682	17.6	488	27.7
Misoprostol BID - III	658	17.6	488	27.7
Misoprostol QD - IV	648	24.9	650	26.1
Misoprostol QD - IV	664	20.2	568	28.1
Misoprostol QD - IV	639	22.2	568	25.6
Misoprostol QD - IV	677	21.5	568	26.4
Misoprostol QD - IV	568	22.8	568	24.9
Misoprostol QD - IV	651	21.5	568	26.4

Mean Total Aspirin Dose for Each Group over Entire Study Length

Treatment - Group Number	Mean Aspirin Dose (mg/kg)
Control - I	26.63
Misoprostol TID - II	26.79
Misoprostol BID - III	27.01
Misoprostol QD - IV	26.25

Appendix Five: Serum Salicylate Concentrations for Individual Dogs Receiving Aspirin Alone or Aspirin with Misoprostol

Treatment - Group Number	Individual Dog Number	Serum Salicylate [b] Concentration (mg/dl)
Control - I	661	12.9
Control - I	689	15.0
Control - I	633	11.6
Control - I	681	11.7
Control - I	663	13.9
Control - I	640	11.8
Misoprostol TID - II	659	19.9
Misoprostol TID - II	656	14.8
Misoprostol TID - II	635	16.7
Misoprostol TID - II	693	15.5
Misoprostol TID - II	672	15
Misoprostol TID - II	646	19.8
Misoprostol BID - III	660	10.3
Misoprostol BID - III	645	11.1
Misoprostol BID - III	650	13.7
Misoprostol BID - III	636	16.1
Misoprostol BID - III	682	12.9
Misoprostol BID - III	658	9.5
Misoprostol QD - IV	648	15
Misoprostol QD - IV	664	12.5
Misoprostol QD - IV	639	15.2
Misoprostol QD - IV	677	13.4
Misoprostol QD - IV	568	14.8
Misoprostol QD - IV	651	9.1

Mean Serum Salicylate Concentration for Each Group

Treatment - Group Number	Mean Serum Salicylate Concentration (mg/dl)
Control - I	12.82
Misoprostol TID - II	16.95
Misoprostol BID - III	12.27
Misoprostol QD - IV	13.33

[b]salicylate quantitation performed using standard fluoroscopic technique (ACA-IV Instrument Manual; E.I. DuPont de Nemours & Company, Inc, Wilmington, DE); all samples obtained by jugular venapuncture on day 22 of study

Appendix Six: Total Dog-Days of Vomiting, Anorexia and Diarrhea for IIndividual Dogs Receiving Aspirin Alone or Aspirin with Misoprostol over the Entire Study Length

Treatment - Group Number	Dog Number	Dog-Days of Vomiting	Dog-Days of Anorexia	Dog-Days of Diarrhea
Control - I	661	0	0	0
Control - I	689	0	0	7
Control - I	633	0	0	5
Control - I	681	0	0	7
Control - I	663	0	0	0
Control - I	640	0	0	0
Misoprostol TID - II	659	0	0	0
Misoprostol TID - II	656	1	0	0
Misoprostol TID - II	635	0	0	15
Misoprostol TID - II	693	0	0	0
Misoprostol TID - II	672	0	0	0
Misoprostol TID - II	646	0	0	5
Misoprostol BID - III	660	0	0	0
Misoprostol BID - III	645	1	0	16
Misoprostol BID - III	650	0	0	2
Misoprostol BID - III	636	1	0	23
Misoprostol BID - III	682	1	0	9
Misoprostol BID - III	658	0	0	0
Misoprostol QD - IV	648	2	0	0
Misoprostol QD - IV	664	7	0	0
Misoprostol QD - IV	639	0	0	0
Misoprostol QD - IV	677	0	0	0
Misoprostol QD - IV	568	3	0	0
Misoprostol QD - IV	651	1	0	11

Mean Dog-Days of Vomiting, Anorexia and Diarrhea for Each Group for Entire Length of Study

Treatment - Group Number	Dog-Days of Vomiting	Dog-Days of Anorexia	Dog-Days of Diarrhea
Control - I	0	0	3.17
Misoprostol TID - II	0.17	0	3.33
Misoprostol BID - III	0.5	0	8.33
Misoprostol QD - IV	2	0	1.83

Appendix Seven: Statistical Analysis

A. Analysis of Day -9 Median Total Gastric Lesion Scores of Dogs Receiving Aspirin and/or Misoprostol Using Kruskal-Wallis Rank Sum Test

Treatment - Group Number	Least Squares Mean	P value (compared to Group I)	P value (compared to Group II)	P value (compared to Group III)	P value (compared to Group IV)
Control - I	12.00		0.1727	1.0000	1.0000
Misoprostol TID - II	14.00	0.1727		0.1727	0.1727
Misoprostol BID - III	12.00	1.0000	0.1727		1.0000
Misoprostol QD - IV	12.00	1.0000	0.1727	1.0000	

*significant difference at $p \leq 0.05$

B. Analysis of Day 5 Median Total Gastric Lesion Scores of Dogs Receiving Aspirin and/or Misoprostol Using Kruskal-Wallis Rank Sum Test

Treatment - Group Number	Least Squares Mean	P value (compared to Group I)	P value (compared to Group II)	P value (compared to Group III)	P value (compared to Group IV)
Control - I	16.75		0.0129*	0.0013*	0.5872
Misoprostol TID - II	8.91	0.0129*		0.3215	0.0037*
Misoprostol BID - III	6.00	0.0013*	0.3215		0.0003*
Misoprostol QD - IV	18.33	0.5872	0.0037*	0.0003*	

*significant difference at $p \leq 0.05$

C. Analysis of Day 14 Median Total Gastric Lesion Scores of Dogs Receiving Aspirin and/or Misoprostol Using Kruskal-Wallis Rank SumTest

Treatment - Group Number	Least Squares Mean	P value (compared to Group I)	P value (compared to Group II)	P value (compared to Group III)	P value (compared to Group IV)
Control - I	13.17		0.3825	0.3387	0.2533
Misoprostol TID - II	9.75	0.3825		0.9314	0.0517*
Misoprostol BID - III	9.41	0.3387	0.9314		0.0434*
Misoprostol QD - IV	17.66	0.2533	0.0517*	0.0434*	

*significant difference at $p \leq 0.05$

D. Analysis of Day 28 Median Total Gastric Lesion Scores of Dogs Receiving Aspirin and/or Misoprostol Using Kruskal-Wallis Rank Sum Test

Treatment - Group Number	Least Squares Mean	P value (compared to Group I)	P value (compared to Group II)	P value (compared to Group III)	P value (compared to Group IV
Control - I	14.58		0.4399	0.0177*	0.3731
Misoprostol TID - II	11.91	0.4399		0.0873	0.1048
Misoprostol BID - III	5.83	0.0177*	0.0873		0.0023*
Misoprostol QD - IV	17.66	0.3731	0.1048	0.0023*	

* significant difference at $p \leq 0.05$

E. Statistical Comparison of Average Dose of Misoprostol for Each Group of Dogs Receiving Aspirin and/or Misoprostol Using Tukey's Honestly Significant Difference Test (HSD)

Alpha = 0.05 df = 20 MSE = 0.010651
Critical Value of Studentized Range = 3.958
Minimum Significant Difference = 0.01668

Treatment - Group Number	Mean	Tukey Grouping
Control - I	0	B
Misoprostol TID - II	3.02	A*
Misoprostol BID - III	3.12	A*
Misoprostol QD - IV	3.01	A*

*Means with the same letter are not significantly different by Tukey's HSD

F. Statistical Comparison of Average Dose of Aspirin for Each Group of Dogs Receiving Aspirin and/or Misoprostol Using Tukey's Honestly Significant Difference Test (HSD)t

Alpha = 0.05 df = 20 MSE = 1.679952
Critical Value of Studentized Range = 3.958
Minimum Significant Difference = 2.0945

Treatment - Group Number	Mean	Tukey Grouping
Control - I	26.63	A*
Misoprostol TID - II	26.79	A*
Misoprostol BID - III	27.01	A*
Misoprostol QD - IV	26.25	A*

*Means with the same letter are not significantly different by Tukey's HSD

G. Statistical Comparison of Average Serum Salicylate Concentration for Each Group of Dogs Receiving Aspirin and/or Misoprostol Using Tukey's Honestly Significant Difference Test (HSD)

Alpha = 0.05 df = 20 MSE = 4.7095
Critical Value of Studentized Range = 3.958
Minimum Significant Difference = 3.5069

Treatment - Group Number	Mean	Tukey Grouping
Control - I	12.82	B*
Misoprostol TID - II	16.95	A
Misoprostol BID - III	12.27	B*
Misoprostol QD - IV	13.33	B*

*Means with the same letter are not significantly different by Tukey's HSD

H. Statistical Comparison of Mean Dog-Days of Vomiting for Each Group of Dogs Receiving Aspirin and/or Misoprostol Using Tukey's Honestly Significant Difference Test (HSD)

Alpha = 0.05 df = 20 MSE = 2.016667
Critical Value of Studentized Range = 3.958
Minimum Significant Difference = 2.2948

Treatment - Group Number	Mean	Tukey Grouping
Control - I	0.000	A*
Misoprostol TID - II	0.167	A*
Misoprostol BID - III	0.500	A*
Misoprostol QD - IV	2.000	A*

*Means with the same letter are not significantly different by Tukey's HSD

I. Statistical Comparison of Mean Dog-Days of Diarrhea for Each Group of Dogs Receiving Aspirin and/or Misoprostol Using Tukey's Honestly Significant Difference Test (HSD)

Alpha = 0.05 df = 20 MSE = 40.01667
Critical Value of Studentized Range = 3.958
Minimum Significant Difference = 10.222

Treatment - Group Number	Mean	Tukey Grouping
Control - I	3.17	A*
Misoprostol TID - II	3.33	A*
Misoprostol BID - III	8.33	A*
Misoprostol QD - IV	1.83	A*

*Means with the same letter are not significantly different by Tukey's HSD

J. Statistical Comparison of Regional Gastric Lesion Score between Endoscopic Stomach Regions for All Groups of Dogs Receiving Aspirin and/or Misoprostol Over Using Tukey Honestly Significant Difference Test (HSD)

Alpha = 0.05 df = 20 MSE = 2.174479
Critical Value of Studentized Range = 3.958
Minimum Significant Difference = 3.5069

Gastric Region	Mean Gastric Lesion Score	Tukey Grouping
Cardia	3.75	A*
Greater Curvature	3.61	A*
Angularis Incisura	3.03	B
Pyloric Antrum	2.49	C

*Means with the same letter are not significantly different by Tukey's HSD

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Vita

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