

GASTROINTESTINAL TRACT

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disorder characterized by inflammation of synovial joints leading to progressive erosion of cartilage and bone. RA is associated with swelling, pain, and stiffness of multiple synovial joints, with an annual incidence of 31 per 100,000 women and 13 per 100,000 men. RA is more common in women than in men by a ratio of approximately 3:1 (Doan and Massarotti, 2005). Osteoarthritis (OA) is a common, age-related disorder of synovial joints that is pathologically characterized by irregularly distributed loss of cartilage more frequently in areas of increased load, sclerosis of subchondral bone, subchondral cysts, marginal osteophytes, increased metaphyseal blood flow, and variable synovial inflammation. Cyclooxygenase (COX) has two distinct membrane-anchored isoenzymes: a constitutively expressed (COX-1) and a highly induced (COX-2) isoenzyme. Following exogenous stimuli (i.e., inflammation), arachidonic acid is liberated by phospholipases. COX-1 and COX-2 are rate-limiting enzymes with COX and peroxidase activities that catalyze the conversion of arachidonic acid to prostaglandin (PG) endoperoxide (PGG₂) and prostanoids, which are then reduced to PGH₂ (Eling et al., 1990). PGH₂ is further metabolized to thromboxane A₂ (TXA₂), prostacyclin (PGI₂), PGD₂, PGF_{2α}, and PGE₂ (Fig. 1-1) (Dannenberg et al., 2001; Radi and Khan, 2006b; Radi, 2009). Prostanoids, including TXA₂ and PGI₂, help regulate vascular tone and thrombosis via COX activity. TXA₂ is a vasoconstrictor that is largely platelet derived and COX-1 dependent, and it promotes platelet adhesion and aggregation and smooth muscle cell proliferation. PGI₂ is an endothelial-derived vasodilator with antiaggregatory platelet functions but is both COX-1 and COX-2 dependent (Kearney et al., 2004).

The inhibitors of COX activity include nonselective nonsteroidal anti-inflammatory drugs (ns-NSAIDs) and COX-2 selective nonsteroidal anti-inflammatory drugs (s-NSAIDs). Nonselective NSAIDs, at therapeutic doses, inhibit both COX-1 and COX-2 (Fig. 1-1) (Dannenberg et al., 2001; Radi and Khan, 2006b; Radi, 2009). The analgesic and anti-inflammatory properties of NSAIDs are linked to COX-2 inhibition, while many of the gastrointestinal tract (GI) toxicities and side effects have been linked variably to COX-1 and/or COX-2 inhibition and, in some cases, directly to the secondary pharmacologic properties of

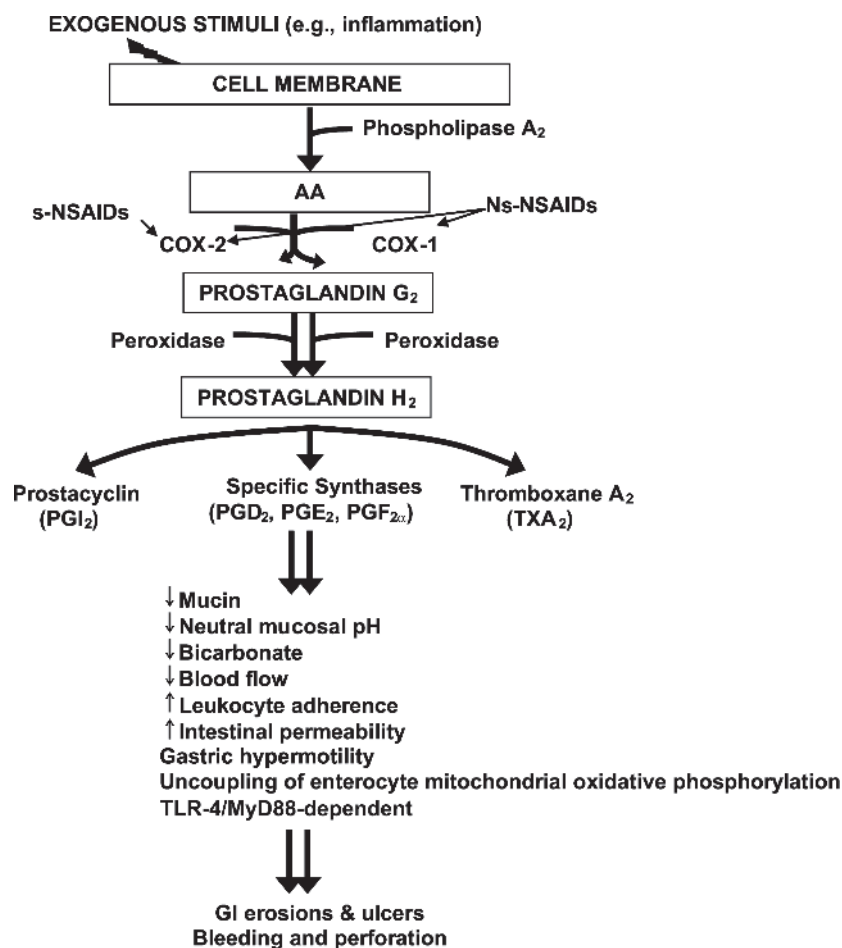


FIGURE 1-1 Pathophysiological role of prostaglandins (PGs) in the gastrointestinal (GI) tract and effects of ns-NSAIDs and COX-2 s-NSAIDs. Following an exogenous stimulus (e.g., inflammation), cell membrane phospholipid is liberated to arachidonic acid (AA) by phospholipase A₂. Both COX-1 and COX-2 catalyze the conversion of AA into various PGs. COX-1 is the predominant isoform in the normal GI tract (gastric fundus, corpus, antrum and/or pylorus, duodenum, jejunum, ileum, cecum, and colon), while COX-2 expression is up-regulated during inflammatory or neoplastic conditions. Nonselective NSAIDs (e.g., carprofen, etodolac, flunixin meglumine, ketoprofen, indomethacin, phenylbutazone) inhibit COX-1 and COX-2, while selective s-NSAIDs (e.g., celecoxib, firocoxib, rofecoxib, lumiracoxib, valdecoxib) spare COX-1 and inhibit only COX-2. Potential mechanisms of ns-NSAID-mediated GI toxicity include (1) increased intestinal epithelial permeability, (2) uncoupling of mitochondrial oxidative phosphorylation, (3) gastric hypermotility, (4) decreased epithelial cell secretion of bicarbonates, (5) decreased mucin secretion, (6) decreased blood flow, (7) decreased neutral pH of mucosa, (8) leukocyte infiltration, and (9) TLR-4/MyD88-dependent into the GI mucosa after injury. Loss of these GI protective mechanisms can lead to GI erosion, ulcers, bleeding, and perforation. (Reprinted from Z. A. Radi and N. K. Khan, *Effects of cyclooxygenase inhibition on the gastrointestinal tract*, *Experimental and Toxicologic Pathology*, 58, pp. 163–173. Copyright © 2006, with permission from Elsevier.)

the select drugs. COX-2 selective NSAIDs (i.e., celecoxib, deracoxib, etoricoxib, firocoxib, lumiracoxib, parecoxib, robenacoxib, rofecoxib, and valdecoxib) were developed to provide a drug that is selective for COX-2, which at therapeutic doses demonstrated therapeutic benefits comparable to those of conventional ns-NSAIDs without the attendant COX-1-mediated toxicities (Radi and Khan, 2006b). The first human-use COX-2 s-NSAIDs were celecoxib and rofecoxib, approved for the treatment of OA and RA. Later drug developments would produce deracoxib, etoricoxib, firocoxib, parecoxib, lumiracoxib, robenacoxib, and valdecoxib. The focus of this chapter is a detailed examination of the comparative expression of COX-1 and COX-2, the effects of COX-2 selective and nonselective NSAID inhibition on the GI system, and the pathophysiological mechanisms of such GI effects and toxicities.

COMPARATIVE COX-1 AND COX-2 EXPRESSION IN THE GI TRACT

GI expression of COX-1 and COX-2 in various species is summarized in Table 1-1 (Radi and Khan, 2006b; Radi, 2009). COX-1 (and not COX-2) is the predominant isoform in the normal GI tract (i.e., gastric fundus, corpus, antrum and/or pylorus, duodenum, jejunum, ileum, cecum, and colon) and is expressed normally in canine, humans, and nonhuman primates. The COX-2 isoform is nearly absent in these species, except in rats and for low levels in the large intestine (Kargman et al., 1996; Seibert et al., 1997; Koki et al., 2002a; Maziasz et al., 2003). Both COX-1 and COX-2 are present in the normal human gastric mucosa and colon (Jackson et al., 2000; Fornai et al., 2006).

COX-1 is found in the mucosal epithelium, vascular endothelium, neurones of myenteric ganglia, and in smooth muscle cells of the tunica muscularis. However, expression levels of COX-1 in the GI tract show wide intra-anatomical and interspecies variability. For example, both the gastric antrum and pyloric region of dogs contain 10-fold more COX-1 protein than is contained in the small intestine

TABLE 1-1 Comparative COX-1 and COX-2 Expression in the Gastrointestinal Tract

Location	COX-1					COX-2				
	Dog	Horse	Human	Monkey	Rat	Dog	Horse	Human	Monkey	Rat
Stomach fundus	×		×	×	×			×		×
Pyloric antrum	×		×	×	×			×		×
Duodenum	×		×	×	×					×
Jejunum	×	×	×	×	×		×			×
Ileum	×		×	×	×					×
Cecum	×		×	×	×					×
Colon	×		×	×	×	×		×	×	×

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(Seibert et al., 1997). In comparison, the nonhuman primate small intestine has five-fold more COX-1 protein than that in rodent or canine small intestine tissues, and rodents express less COX-1 in the GI tract than do nonhuman primates or humans (Kargman et al., 1996). In rats, weak COX-1 immunostaining is found in the stomach, small intestine, and colon (Burdan et al., 2008). In a rat model of colitis, both COX-1 and COX-2 were expressed in the normal colon and present in neurons of myenteric ganglia, while COX-2 was up-regulated in rats with colitis (Fornai et al., 2006). In rabbits, only COX-1 was detected in parietal cells, while both COX-1 and COX-2 were expressed in gastric glands, with the relative protein density of COX-1 being sixfold higher than that of COX-2 (Nandi et al., 2009). In humans, the highest and lowest areas of COX-1 expression are in the small intestine and gastric fundus/antrum, respectively (Kargman et al., 1996). Strong gastric parietal cell COX-1 and COX-2 immunoreactivity has been observed in the normal human gastric mucosa (Jackson et al., 2000). COX-2 up-regulation has been described within the mucosa in the presence of inflammation or ulcers. COX-1 and COX-2 immunostaining was increased at the rim of ulcers and in *Helicobacter pylori* gastritis, particularly at the mid-glandular zone and lamina propria inflammatory cells (Jackson et al., 2000). Some studies suggest that the predominant source of increased gastric PGE₂ in *H. pylori* infection in humans is probably COX-1 derived (Scheiman et al., 2003). In inflammatory bowel disease (IBD), COX-1 was localized in the crypt epithelium of the normal ileum and colon and its expression was unchanged. COX-2 expression, on the other hand, was undetectable in normal ileum or colon but was induced in apical epithelial cells of inflamed foci in IBD (Singer et al., 1998). In another study, COX-2 expression up-regulation occurred in neural cells of the myenteric plexus in patients with active IBD (Roberts et al., 2001).

COX-2 is normally absent (except in the colonic mucosa) in the intestinal tract in dogs, nonhuman primates, and humans (Koki et al., 2002a; Maziasz et al., 2003). In horses, COX-1 and COX-2 were expressed in nonischemic- and ischemic-injured jejunal mucosa tissues obtained 18 h after recovery, with ischemia causing significant up-regulation of both COX isoforms (Tomlinson et al., 2004). In rats, COX-2 is present at low levels in close association with macrophages in the region of gut-associated lymphoid tissue (Kargman et al., 1996). COX-2 expression was observed in the rat fundus and pylorus regions of the stomach, intestinal tract (jejunum, ileum, duodenum, cecum, colon, and rectum), and intestinal tract parasympathetic ganglia of the submucosa and muscularis (Haworth et al., 2005). The highest level of COX-2 expression was noted at the ileocecal junction in rats (Haworth et al., 2005). This ileal-side high level of COX-2 expression may explain the spontaneous ulceration and perforation of the distal ileum in COX-2 knockout (COX-2^{-/-}) rodents (Sigthorsson et al., 2002). There is site-dependent susceptibility to intestinal injury that is related to local prostanoid homeostasis. For example, the rat cecum is particularly sensitive to long-term, low-dose indomethacin administration (NygAard et al., 1995). COX-2 immunostaining was observed in the small intestine lamina propria in mice (Hull et al., 1999).

COX-2 can be induced in pathological conditions and in the inflamed GI mucosa, and its inhibition by NSAIDs has been hypothesized to delay the resolution of GI injury (Kishimoto et al., 1998). Increased COX-2 expression, observed

TABLE 1-2 Comparative Susceptibility to Toxicity and Location^a of Lesions in the Gastrointestinal Tract After COX Inhibition

GI injury	Interspecies differences
Relative susceptibility at therapeutic exposures	Rat > dog > monkey > human
Upper GI most common site	Human > monkey > dog > rat
Lower small intestine most common site	Rat > dog > monkey ^b > human

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^aLocation of injury similar for both ns-NSAIDs and s-NSAIDs, with the exception that injuries with s-NSAIDs occur at high exposure multiples.

^bInjury to lower GI tract uncommon in monkeys.

maximally at 24 h, has been observed in a rat model of ischemia/reperfusion–induced acute gastric mucosal injury (Kishimoto et al., 1998). COX-2 expression was increased in cultured rat gastric mucosal cells in vitro and after acid-induced gastric injury to rats in vivo (Sawaoka et al., 1997; Erickson et al., 1999; Sun et al., 2000). COX-2 may play a role in rodent postoperative ileus since intestinal manipulation induced COX-2 within resident muscularis macrophages, a discrete subpopulation of myenteric neurons and recruited monocytes (Schwarz et al., 2001). Additionally, COX-2 has been shown to be induced in various hyperplastic and neoplastic lesions of the GI tract, such as colon cancer, familial adenomatous polyposus (FAP), and sporadic adenomatous polyps in the colon (Soslow et al., 2000; Khan et al., 2001; Koki et al., 2002b). Up-regulation of COX-2 has been demonstrated within adenomas of the small and large intestine of multiple intestinal neoplasia (Min) mice (Hull et al., 1999). These observations support the use of NSAIDs in the treatment of epithelial cancer. In fact, a COX-2 s-NSAID, celecoxib, has been approved as adjunct therapy to the usual care (e.g., endoscopic surveillance, surgery) to reduce the number of adenomatous colorectal polyps in humans. In humans, gastric mucosal expression of COX-2 is increased in gastritis and gastric ulceration (Tatsuguchi et al., 2000; Bhandari et al., 2005).

In summary, COX-1 (and not COX-2) is the predominant isoform in the normal GI tract. There appears to be significant interspecies differences in both the level of COX-1 expression and the ratio of COX-1 and COX-2 expression in the GI tract. Both COX-1 expression and relative COX-1/COX-2 expression are highest in some animal species, including the dog and rat, compared with humans and nonhuman primates, which may partly explain the overt sensitivity of these species to sub-therapeutic doses of ns-NSAIDs (Table 1-2) (Radi and Khan, 2006b; Radi, 2009).

EFFECTS OF ns-NSAIDS ON THE GI TRACT

Several ns-NSAID classes (Table 1-3) are used in human and veterinary medicine for their anti-inflammatory, analgesic, and antipyretic effects. In veterinary medicine, phenylbutazone, meclofenamic acid, meloxicam, carprofen, and

TABLE 1-3 Nonselective NSAID Major Classes

ns-NSAID class	Generic name	Trade name
Arylpropionic acid	Ibuprofen	Advil, Motrin, Nuprin
	Naproxen	Aleve, Naprosyn
	Ketoprofen	Orudis, Oruvail
	Carprofen	Rimadyl, Zenecarp, Novox
	Fenoprofen	Nalfon
	Flurbiprofen	Flurofen, Ansaïd
Enolic acids	Piroxicam	Feldene
	Tenoxicam	Tilcotil
	Lornoxicam	Xefo
Acetic acids	Etodolac	Etogesic
	Indomethacin	Indocin
	Diclofenac	Cataflam, Voltarin
	Sulindac	Clinoril
	Nabumetne	Relfen
Aminonicotinic acids	Flunixin meglumine	Banamine
Pyrazoles	Phenylbutazone	Zolandin
Salicylic acids	Acetylsalicylic acid	Aspirin
Anthranilic acids	Meclofenamate	Ponstel, Arquel, Meclofen

etodolac are approved for use in dogs in the United States (Fox and Johnston, 1997; Budsberg et al., 1999); flunixin meglumine, meclufenamic acid, naproxen, and phenylbutazone are approved for horses (Kopcha and Ahl, 1989). Although the broad range of applications for ns-NSAID therapy makes it an attractive prescriptive choice, it has been partnered adversely with GI toxicity. Implications to humans have included nonulcer dyspepsia and serious GI-related side effects, such as gastric and duodenal ulcers, erosions, bleeding, perforation, esophagitis, and esophageal strictures (Lanza et al., 1983; Bjorkman, 1996; Mason, 1999; Schoenfeld et al., 1999; Scheiman, 2003). Similar to use in humans, chronic ns-NSAID use in dogs has also been associated with serious GI side effects, manifested as bleeding, ulceration, erosions, perforations, peritonitis, melena, anemia, anorexia, and abdominal pain (Ewing, 1972; Roudebush and Morse, 1981; Cosenza, 1984; Daehler, 1986; Stanton and Bright, 1989; Wallace et al., 1990; Ricketts et al., 1998; Reed, 2002). The incidence of GI ulceration is greatly increased in animals receiving ns-NSAIDs in combination with steroids; therefore, this combination should be avoided (Johnston and Budsberg, 1997; Reed, 2002).

To limit the potential for serious complications associated with NSAID use in veterinary medicine, clinicians should take the following precautions before prescribing NSAIDs: (1) verify that corticosteroids and other NSAIDs are not being given concurrently with the NSAID prescribed, (2) adhere to the dosages recommended, (3) advise clients of potential safety risks and their clinical signs,

and (4) avoid use in at-risk cases (Lascelles et al., 2005b). At-risk indications are (1) a history of GI ulceration, (2) geriatric patients (older animals have reduced clearance capacity and are more susceptible to NSAID GI toxicity), (3) the use of aspirin, (4) GI comorbidities (e.g., preexisting GI ulcer, *H. pylori* colonization, liver disease), and (5) clinical chemistry (e.g., indications of impaired hepatic function, hypoproteinemia) (Lascelles et al., 2005b). The comparative GI effects of various classes of ns-NSAIDs are detailed below.

Effects of Arylpropionic Acid ns-NSAIDs on the GI Tract

Arylpropionic acids represent the largest class of widely prescribed group of ns-NSAIDs, which include ibuprofen, naproxen, ketoprofen, carprofen, fenoprofen, and flurbiprofen (Table 1-3). These drugs are approved for use in the treatment of RA, OA, and ankylosing spondylitis.

Ibuprofen (Advil, Motrin, and Nuprin) is one of the most commonly used ns-NSAIDs and is supplied as tablets. It probably ranks after aspirin and paracetamol in nonprescription over-the-counter (OTC) drugs used in humans for the relief of symptoms of pain, inflammation, and fever (Rainsford, 2009). In dogs, ibuprofen has been used as an anti-inflammatory agent. However, dogs are much more sensitive than humans to the development of GI toxicity from ibuprofen administration. At therapeutic doses, adverse GI effects observed in dogs include vomiting, diarrhea, anorexia, abdominal pain, nausea, and GI bleeding. Dogs given 8 or 16 mg/kg per day of ibuprofen orally for 30 days showed no clinical signs of toxicity. However, postmortem examination revealed the presence of gastric ulcers or erosions, usually in the antrum or pylorus, less often in the fundic or cardiac regions of the stomach, and intestinal inflammation (Adams et al., 1969). No GI lesions were noted in a 4-mg/kg per day dose in this study in dogs. In another study, ibuprofen oral repeated dosing at 8 and 16 mg/kg per day (0.4- and 0.9-fold multiples of the human dose, respectively) for one month in dogs caused GI pathology comprised of bloody or discolored stools and intestinal ulceration and/or perforation (Hallesy et al., 1973). During 26-week repeat-dose toxicity in dogs given ibuprofen in a 16-mg/kg per day dose, clinical signs of GI toxicity characterized by frequent vomiting, diarrhea with occasional passage of fresh blood, and loss of weight were noted in week 8 of dosing (Adams et al., 1969). Dogs given 4- and 2-mg/kg per day doses had no evidence of GI toxicity. On the other hand, similar GI pathology was observed in rats only after six months of repeated dosing and at a higher oral dose of 180 mg/kg per day (9.7-fold multiples of the human dose) (Hallesy et al., 1973). The approximate LD₅₀ values for ibuprofen are 800 mg/kg orally and 320 mg/kg intraperitoneally in the mouse and 1600 mg/kg orally and 1300 mg/kg subcutaneously in the rat (Adams et al., 1969). The proportions of the dose recovered from the ligated stomach and ligated intestine of rats at various times after introduction of ¹⁴C-labeled ibuprofen were measured. Only 73% of the dose was recovered from the stomach and its contents 3 min after dosing, while no radioactivity was detected in plasma (Adams et al., 1969). Intestinal absorption of ibuprofen in rats was so rapid that by 3 min the plasma concentration of radioactivity was maximal (Adams et al., 1969). Thus, although

some absorption occurs in the stomach, the main site of ibuprofen absorption, at least in rats, is the intestine. Rats given ibuprofen for 26 weeks orally at 180 mg/kg per day grew normally but were anemic (had low erythrocyte counts, hemoglobin concentration, and hematocrits) by the final week of dosing, and a few rats had intestinal ulcers (Adams et al., 1969). Therefore, due to its narrow margin of safety, ibuprofen is generally not recommended for use or should be used with caution in dogs and also in cats or ferrets (Cathers et al., 2000). Ibuprofen toxicosis can occur in dogs and ferrets after accidental ingestion. When ibuprofen toxicosis is suspected, serum, urine, and liver samples can be used for toxicological analyses for ibuprofen by gas chromatography and mass spectrophotometry (Cathers et al., 2000). In laboratory animals, ibuprofen administration has been linked to vomiting, gastric irritation, and ulceration and duodenal and jejunal ulceration in rats, dogs, rabbits, and monkeys (Adams et al., 1969; Scherkl and Frey, 1987; Elliott et al., 1988; Godshalk et al., 1992; Arai et al., 1993). Pregnant female rabbits given 60 or 20 mg/kg per day of ibuprofen on days 1 to 29 of pregnancy grew less than controls and had stomach ulcers (Adams et al., 1969). Female rabbits receiving 7.5 mg/kg per day grew normally, but some had gastric ulcers or erosions (Adams et al., 1969). Thus, pregnant rabbits are highly sensitive to ibuprofen, and during pregnancy the intestinal tract, at least in rabbits, is more sensitive than that of nonpregnant animals (Adams et al., 1969). The route of ibuprofen administration affects the GI pathology observed. Little or no GI damage occurred when ibuprofen was given daily by oral administration to nonhuman primates at doses up to 300 mg/kg for 90 days. However, intravenous (IV) administration of ibuprofen to nonhuman primates over a 24-h period in four equal doses of 75 mg/kg at 6-h intervals resulted in gastric erosions or ulcers (Elliott et al., 1988). When given IV for 14 days at 100 and 200 mg/kg per day using the same 24-h dosing conditions described above, nonhuman primates showed gastric and/or duodenal ulcers (Elliott et al., 1988). In rats, acute single oral doses under 500 mg/kg of ibuprofen were free of GI pathological changes (Elliott et al., 1988). However, acute IV administration of ibuprofen at a dose of 270 mg/kg given in four equal doses of 67.5 mg/kg at 6-h intervals over a 24-h period resulted in gastric and intestinal (ileum and jejunum) ulcerations (Elliott et al., 1988). Therefore, the acute (24 h) IV route of ibuprofen administration in rats is more ulcerogenic than the oral route.

One factor that has been correlated with GI events with ns-NSAID use is the drug plasma elimination half-life ($t_{1/2}$). There is less gastric mucosal adaptation with NSAIDs that have long half-lives. For example, due to the short plasma $t_{1/2}$ of elimination of ibuprofen, approximately 2 h, and its rapid absorption, ibuprofen at doses of 200 and 800 mg/kg has low possibilities of serious GI events and complications (i.e., epigastric or abdominal pain, dyspepsia, flatulence, nausea, heartburn, diarrhea, constipation, vomiting) in humans (Rainsford, 2009). Another GI event that can be associated with ns-NSAID intake is chronic anemia. For example, daily treatment (800 mg three times daily) with ibuprofen has also been associated with significant fecal blood loss in healthy volunteers (Bowen et al., 2005). Several ns-NSAIDs, including ibuprofen, were compared for GI events in a large two-year epidemiological safety study involving 30,000 to 40,000 rheumatic patients in centers in Germany, Switzerland, and Austria, known as the Safety Profile of

Antirheumatics in Long-Term Administration (SPALA) (Rainsford, 2009). This SPALA study found ibuprofen to be associated with the lowest numbers of GI events. In another large-scale study, fewer GI events were observed in patients taking ibuprofen at doses up to 1200 mg daily for 7 days compared with aspirin and paracetamol (Rampal et al., 2002).

Similarly, naproxen (Aleve, Naprosyn), which has a variably longer half-life across species ($t_{1/2}$ is approximately 14 h in humans, 2 h in nonhuman primates, 35 h in dogs, 9 h in guinea pigs, and 5 h in rats), is used in humans and dogs for its anti-inflammatory, analgesic, and antipyretic properties (Hallesy et al., 1973; Rainsford, 2009). The long half-life of naproxen in dogs appears to be due to its extensive enterohepatic recirculation. With the exception of the dog, all species excreted naproxen and its metabolic transformation products predominantly in the urine. In dogs, naproxen is eliminated primarily through the bile and feces, whereas in other species, the primary route of elimination is through the kidneys (Runkel et al., 1972). Once in the blood after oral administration, naproxen is absorbed fully and rapidly in all species. Naproxen is indicated for temporary relief of fever and minor aches and pains due to backache, headache, and toothache in humans (Runkel et al., 1972; Rainsford, 2009). In dogs, naproxen has been shown to cause gastric ulceration and hemorrhage, melena, vomiting, abdominal pain, weakness, and hemorrhagic gastroenteropathy, including transmural pyloric perforation (Daehler, 1986; Stanton and Bright, 1989). Repeated oral administration of naproxen to dogs for one month at 15 mg/kg per day (1.3-fold multiple of human dose) and for three months at 5 mg/kg per day (0.4-fold multiples of human dose) caused GI pathology of bloody or discolored stools and intestinal ulceration and/or perforation (Hallesy et al., 1973). Additionally, mice and rats administered an acute oral dose of naproxen displayed bloody or discolored stools and intestinal ulceration and perforation (Rainsford et al., 2003). A single oral naproxen dose of 250 mg/kg to rats caused death in 6 to 8 days and abdominal adhesions, and small intestine necrotic foci were observed at necropsy (Elliott et al., 1988). Repeated oral administration of naproxen to rats for six months at 30 mg/kg (2.6-fold multiple of human dose) and for 22 months at 2, 10, and 30 mg/kg per day (0.2-, 0.9-, and 2.6-fold multiples of human dose, respectively) caused GI pathology of bloody or discolored stools and intestinal ulceration and/or perforation (Hallesy et al., 1973). Repeated oral administration of naproxen in nonhuman primates for six months at doses up to 120 mg/kg per day (10.4-fold multiples of human dose) was well tolerated with no adverse GI pathological toxicity (Hallesy et al., 1973). The pig closely resembles humans in respect to anatomy, physiological functions in the GI tract, and the histological and pathophysiological changes in the development of gastric ulcers induced by NSAIDs (Rainsford et al., 2003). Daily oral administration of naproxen to pigs at doses up to 45 mg/kg (3.9-fold multiples of human dose) for one year was well tolerated with no adverse GI pathology (Hallesy et al., 1973). In an experimental pig model using healthy Landrace males, naproxen induced gastroduodenal ulcers and erosions when given orally for 10 days at a dose of 100 or 150 mg/kg per day (Rainsford et al., 2003). In horses, naproxen has been used for the treatment of inflammatory conditions and pain from myositis and soft tissue injuries, and it has a reasonable margin of safety. However, adverse GI ulceration has been reported in

horses (Lees and Higgins, 1985). In humans, GI events associated with naproxen include GI erosions and ulcers, dyspepsia, upper abdominal pain, nausea, diarrhea, constipation, abdominal distension, and flatulence (Lohmander et al., 2005).

Ketoprofen (Orudis, Oruvail) has a $t_{1/2}$ of approximately 8.5 h and is used to treat RA in humans (Rainsford, 2009). It is used in dogs, cats, and horses to treat postsurgical and musculoskeletal pain, colic, synovitis, and OA. Its $t_{1/2}$ in dogs and cats is approximately 2 to 3 h and 2 h in horses. Ketoprofen was ulcerogenic when used in laboratory animal models (Rainsford, 1977) but to a lesser degree than other ns-NSAIDs (phenylbutazone and flunixin meglumine). Similarly, in horses, ketoprofen was less toxic than phenylbutazone and flunixin meglumine (MacAllister et al., 1993). Gastric-duodenal erosion and/or hemorrhage and ulceration of the glandular and nonglandular portions of the stomach were noted in dogs and horses, respectively (MacAllister et al., 1993; Forsyth et al., 1998). Similar ulceration was seen in a one-month oral toxicity study with ketoprofen in rats and dogs (Julou et al., 1976). In a study of Sprague–Dawley rats given a 10-mg/kg dose of ketoprofen subcutaneously that had undergone ovariectomy, many rats died or were euthanized within 3 to 7 days after surgery, due to clinical illness that was related to GI ulceration (Lamon et al., 2008). The safety profile of a reduced dosage of ketoprofen (0.25 mg/kg per day) was evaluated in a 30-day oral study in healthy beagle dogs. Mild to moderate gastric mucosal injuries, especially in the pyloric antrum, were observed in this study (Narita et al., 2006). Gastric lesions were observed in a long-term (up to 90 days) study after oral administration of various ns-NSAIDs (i.e., carprofen, etodolac, flunixin meglumine, and ketoprofen) in dogs (Luna et al., 2007). In addition, the bleeding time was significantly longer by day 7 in dogs treated with meloxicam, ketoprofen, and flunixin meglumine (Luna et al., 2007). Scarring in the pyloric antrum suggestive of ulceration healing was present in one of 12 monkeys following 12 months of ketoprofen treatment (Julou et al., 1976).

Interestingly, a study in hamster cheek pouch microcirculation showed that topically applied ketoprofen lysine salt significantly inhibited both the leukocyte adhesion and microvascular leakage induced by bradykinin (Daffonchio et al., 2002). A kallikrein-kinin cascade such as bradykinin has been shown to be involved in gastric ulcers (Sawant et al., 2001). Therefore, this study by Daffonchio et al. suggests that in addition to COX inhibition, ketoprofen may have an antagonistic effect on bradykinin, which may contribute to its ulcerogenic potential. In humans, ketoprofen is often used in a once-daily 200-mg sustained-release formulation to treat rheumatic diseases, especially in elderly patients. Long-term safety and prospective studies on 20,000 patients showed that ketoprofen is associated with a 28% rate of such GI events as peptic ulcers, bleeding, melena, and black stools (Le Loet 1989; Schattenkirchner, 1991). Most of these serious GI side effects occurred during the first three months of treatment.

Carprofen (Rimadyl, Zenecarp, Novox) is approved for use in dogs to treat pain and inflammation associated with OA and pain associated with soft tissue or orthopedic surgery. The $t_{1/2}$ in dogs is approximately 8 h and is highly variable in cats (20 ± 16 h). In dogs, biliary secretion predominates, and 70% of an IV dose of carprofen is excreted in the feces, while 8 to 15% of the dose is excreted

in the urine. In rats, fecal excretion due to biliary secretion varies from 60 to 75%, and urinary excretion accounts for 20 to 30% of an IV dose (Rubio et al., 1980). Therefore, excretion in dogs, rats, and cattle is mainly fecal after biliary secretion, whereas it is primarily urinary in horses. In dogs, most carprofen is metabolized by direct conjugation to an ester glucuronide followed by oxidation to phenol and further conjugation. These conjugated phenols are eliminated in the feces. Carprofen has produced GI lesions that are mild but of no clinical relevance or significance compared with placebos (Reimer et al., 1999). Typical adverse GI effects of this drug include vomiting, diarrhea, and change in appetite (Raekallio et al., 2006). A transient decrease in serum protein and albumin concentrations (concentrations were lower in treated dogs than in those that received placebo at 4 weeks, but not at 8 weeks) was observed after daily administration of carprofen in a two-month study in dogs (Raekallio et al., 2006). When administered orally daily in a 4-mg/kg dose, carprofen induced the lowest frequency of adverse GI effects compared with etodolac, flunixin meglumine, ketoprofen, and meloxicam in a 90-day study in dogs (Luna et al., 2007).

GI ulceration and bleeding are sometimes accompanied secondarily by anemia and hypoproteinemia, due to blood and protein loss (Adams et al., 1969; Lanas et al., 2003). In a 14-day safety study (according to the Rimadyl package insert) involving oral administration of 10 mg/lb twice daily (10 times the recommended total daily dose), two of eight dogs exhibited hypoproteinemia (hypoalbuminemia). Three incidents of black or bloody stool were observed in one dog. Five of eight dogs exhibited reddened areas of duodenal mucosa on gross pathological examination. Histological examination of these areas revealed no evidence of ulceration but did show minimal congestion of the lamina propria in two of the five dogs. In separate safety studies lasting 13 and 52 weeks, respectively, dogs were administered orally up to 11.4 mg/lb per day (5.7 times the recommended total daily dose of 2 mg/lb) of carprofen. In both studies the drug was well tolerated clinically by all the animals. No gross or histological changes were seen in any of the animals treated.

In cats, carprofen is an effective analgesic for soft tissue and orthopedic procedures and is approved in several countries (Australia, France, Germany, United Kingdom) for use at 4 mg/kg for daily subcutaneous or intravenous administration (Steagall et al., 2009). Carprofen was well tolerated, and no clinical or endoscopic adverse GI effects were seen in cats after its administration in clinical trials for up to 5 days (Möllenhoff et al., 2005; Steagall et al., 2009). Although carprofen is not used routinely in nonhuman primates for postoperative analgesia, a dose of 2.2 mg/kg carprofen intramuscularly, or a combination of 0.01 mg/kg buprenorphine and 2.2 mg/kg carprofen intramuscularly provided more reliable postoperative analgesia than did buprenorphine alone (Allison et al., 2007). Although carprofen has been used to treat mastitis in cattle, it is not generally recommended for use in large animals, due to its long $t_{1/2}$ (30 to 40 h).

Fenoprofen (Nalfon) has a relatively short, 3-h half-life. GI events are similar to those with naproxen or ibuprofen. In rats, single fenoprofen oral doses of 1000 to 1600 mg/kg resulted in death and small intestine necrosis and abdominal adhesions (Elliott et al., 1988). Flurbiprofen (Flurofen, Ansaid) caused abdominal adhesions

and small intestinal necrosis or ulceration in rats after either acute oral (at 80 and 125 mg/kg) or intraperitoneal (at 125, 320, and 500 mg/kg) administration (Elliott et al., 1988). Chronic administration in a three-month study in rats caused ulcerative gastritis in 4- and 8-mg/kg doses and 0.5-, 2-, and 4-mg/kg doses in another two-year study (Elliott et al., 1988).

Effects of Enolic Acid (Oxicam) ns-NSAIDs on the GI Tract

Piroxicam (Feldene) is one of few enolic acid derivatives (Table 1-3) that is absorbed completely after oral administration and that undergoes enterohepatic recirculation. Due to its antitumor activity, it is used in dogs and cats to treat some cancers, such as transitional cell carcinoma (TCC) and oral squamous cell carcinoma. The average estimated $t_{1/2}$ is approximately 40 h in dogs and 12 h in cats. Due to this long $t_{1/2}$ in dogs, the steady state is typically not reached for 7 to 12 days. GI irritation was seen in some dogs after bladder TCC treatment with piroxicam orally in a 0.3-mg/kg dose (Knapp et al., 1994). Gastric ulcers occurred in rats after once-daily piroxicam administration in doses of 2.7, 5.3, and 6.7 mg/kg, which are equieffective for indomethacin (10, 20, and 25 mg/kg) (Aguwa, 1985). Gastric ulcers were induced in rats after two oxicam oral dosing. However, the incidence of such lesions was higher for tenoxicam (Tilcotil) (10.2 mg/kg) than for diclofenac sodium (34 mg/kg, equivalent to 6.8 mg/kg tenoxicam) or piroxicam (6.2 mg/kg) (al-Ghamdi et al., 1991). Other subchronic 14- and 28-day studies in rats assessed the GI effects of equipotent doses of meloxicam (3.75 and 7.5 mg/kg) and piroxicam (5 and 10 mg/kg) in rats. Both drugs dose-dependently caused multiple gastric erosions and hemorrhage. Meloxicam led to greater gastric damage than with piroxicam on day 14, although these results were not significant (Villegas et al., 2002). In a dose-escalation study of piroxicam with oral doses ranging from 0.5 mg/kg every 48 h to 1.5 mg/kg every 48 h in dogs, a dose-limiting GI irritation or ulceration occurred in dogs that received 1.5 mg/kg, with a maximum tolerated dose of 1 mg/kg (Knapp et al., 1992). Lornoxicam (Xefo), a novel ns-NSAID compound in the same chemical class as piroxicam and tenoxicam, caused GI lesions in monkeys (Atzpodien et al., 1997). In the dose-range-finding study, animals were dosed orally for 6 weeks with 0.25, 0.5, 1, or 2 mg lornoxicam/kg per day. GI toxicity was observed in the 1- and 2-mg/kg per day dose groups only. Toxicity included mortality, diarrhea, prostration, decreased body weight gain and food consumption, fecal occult blood, anemia, hypoalbuminemia, GI erosions, and ulcerations (Atzpodien et al., 1997). A follow-up chronic study was conducted using dose levels of 0.125, 0.25, or 0.5 mg/kg per day for 52 weeks. The high-dose level was increased to 0.6 mg/kg/day from week 39 to week 52. Histopathological examination of the GI tract revealed erosions, ulcerations, and inflammation in both males and females at 0.5 or 0.6 mg/kg per day. Cinicopathological findings included decreased hematocrit and hypoproteinemia and hypoalbuminemia (Atzpodien et al., 1997).

In a clinical study in elderly patients with knee OA, piroxicam at a dose of 20 mg/day for 3 weeks resulted in elevation of the gastric mucosa endoscopic score in 78% of the subjects compared to the beginning of the study, and 22% of

the subjects developed ulcers. Mild dyspepsia symptoms after piroxicam administration were positive in 67% of subjects (Girawan et al., 2004). Significantly higher bleeding was found in a 28-day study in healthy male volunteers using a 20-mg piroxicam dose compared with a placebo. In addition, endoscopy scores were significantly higher with piroxicam than in the meloxicam group at a dose of 7.5 mg (Patoia et al., 1996). In another 28-day study in healthy volunteers, significant macroscopic gastric mucosal damage occurred within 24 h of 20-mg piroxicam administration; however, such GI damage resolved in most subjects by day 28 (Lipscomb et al., 1998).

Effects of Acetic Acid Derivative ns-NSAIDs on the GI Tract

Acetic acid derivatives include etodolac, indomethacin, diclofenac, sulindac, and nabumetone (Table 1-3). Etodolac (EtoGesic) is approved for use in dogs with OA and has been studied in horses. Adverse reactions to etodolac in dogs include vomiting, soft or dark brown stool, and diarrhea with blood, as reported in a three-month oral toxicity study at a dose of 25 mg/kg, as well as gastric and small intestinal ulceration with associated weight loss, anorexia, anemia, and hypoproteinemia in a one-year chronic toxicity study at doses of 40 and 80 mg/kg (Budsberg et al., 1999). In a 28-day study in healthy dogs, etodolac was given orally once a day at an average dose of 12.8 mg/kg and gastroduodenal endoscopy was performed. Only minor gastric lesions were observed (Reimer et al., 1999). In an experimental study of the GI effects of etodolac in horses, jejunum was exposed to 2 h of ischemia during anesthesia, and then horses received etodolac at 23 mg/kg IV every 12 h. Tissue specimens were obtained from ischemic-injured and nonischemic jejunum immediately after ischemia and 18 h after recovery from ischemia. The investigators found that ischemic-injured tissue from horses treated with etodolac had significantly lower transepithelial electric resistance and retarded recovery of the jejunal mucosa barrier after 18 h of reperfusion (Tomlinson et al., 2004).

In rats, indomethacin (Indocin) caused gastric mucosal bleeding, cecal ulceration, and small intestine (jejunum and ileum) ulcers, perforations, and adhesions (Kent et al., 1969; Brodie et al., 1970; Schriver et al., 1975; Fang et al., 1977; Arai et al., 1993; Anthony et al., 1994; Sigthorsson et al., 1998; Campbell and Blikslager, 2000; Altinkaynak et al., 2003; Takeuchi et al., 2004), with gastric damage being significantly greater in arthritic rats than in normal rats (McCafferty et al., 1995). The half-life of indomethacin in plasma ranges from hours in rats to minutes in dogs and monkeys. There are significant species differences in the distribution and excretion of indomethacin (Yesair et al., 1970). In rats, plasma clearance of indomethacin by liver, although low, is 30 times the clearance rate by kidney, and the reabsorption of indomethacin from the intestine is extensive. Desmethylin-domethacin, the major metabolite, is cleared from plasma equally by liver and kidney and is not reabsorbed from the intestine of rats. In dogs, indomethacin is secreted in bile extensively and rapidly, and is eventually excreted in their feces as an unchanged drug and minimally metabolized to eschlorobenzoylindomethacin, which is excreted in urine. Nonhuman primates are similar to dogs in that the liver was more than 10 times as effective as the kidneys in clearing total radioactivity

from plasma. However, the primates differed from dogs in that the drug was maximally reabsorbed from the intestine (Yesair et al., 1970). In a six-month repeat oral daily dosing study in rats, indomethacin at doses of 2 and 4 mg/kg (0.7- and 1.3-fold multiples over human dose, respectively) caused GI pathology characterized by bloody or discolored stools and intestinal ulceration and/or perforation (Hallesy et al., 1973). Indomethacin increased the incidence and ulcer index of duodenal ulcers in arthritic rats on days 14 and 28 of arthritis (DiPasquale and Welaj, 1973). Additionally, the intestinal ulcerogenic response to indomethacin was markedly aggravated in arthritic rats, and the onset of the ulceration was much earlier in arthritic rats than in normal rats (Kato et al., 2007). GI hemorrhage and ulceration, potentially attributed to the extensive enterohepatic recirculation of indomethacin, were seen in dogs (Duggan et al., 1975) (Figs. 1-2 and 1-3). In a one-month repeat oral dosing study in dogs, indomethacin at 6 and 18 mg/kg per day (1.9- and 5.8-fold multiples over human dose) caused GI pathology of bloody or discolored stools and intestinal ulceration and/or perforation (Hallesy et al., 1973). In an experimental pig model for human GI disease, indomethacin was given orally for 10 days at a

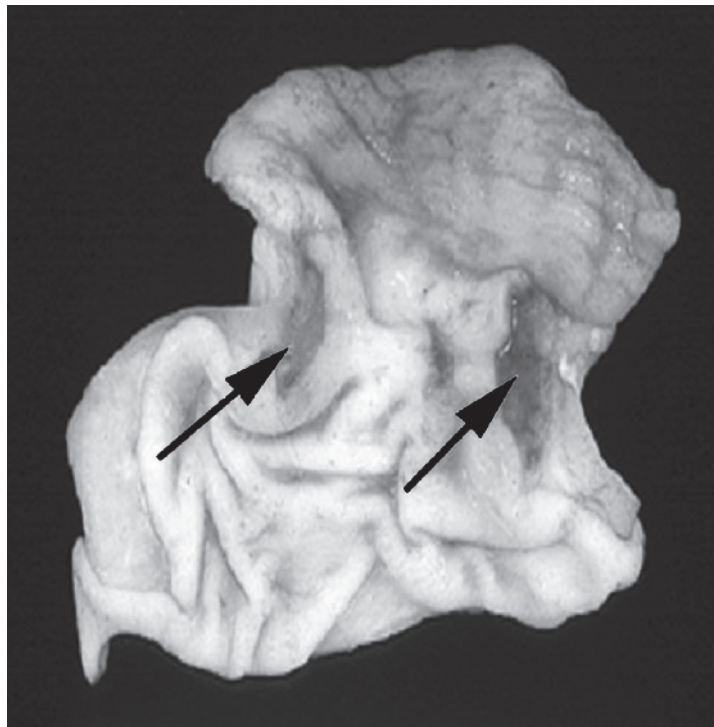


FIGURE 1-2 Severe indomethacin-induced gastric mucosal hemorrhage and ulceration at the gastroduodenal junction (arrows) in a dog. (Reprinted from Z. A. Radi and N. K. Khan, Effects of cyclooxygenase inhibition on the gastrointestinal tract, *Experimental and Toxicologic Pathology*, 58, pp. 163–173. Copyright © 2006, with permission from Elsevier.)

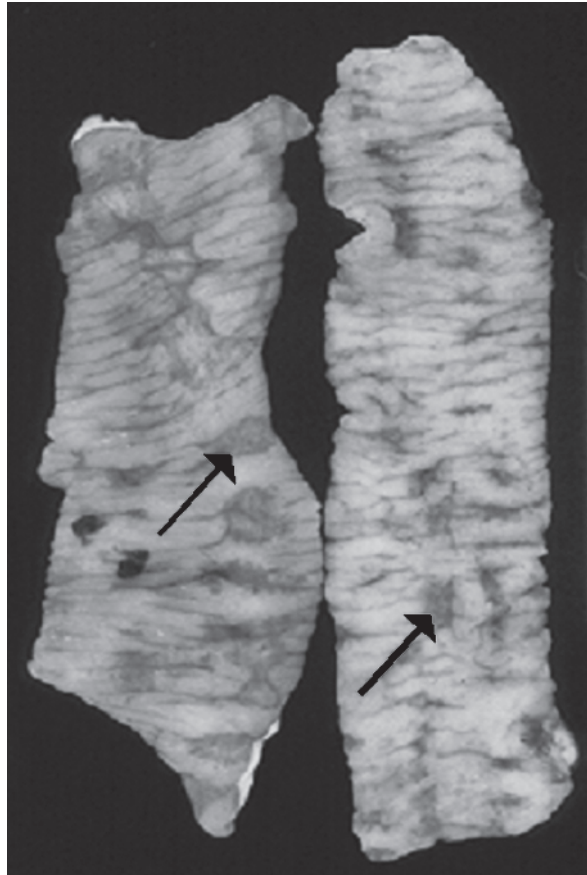


FIGURE 1-3 Indomethacin-induced small intestine mucosal ulceration (arrows) in a dog. Note the skip ulcerations typical of ns-NSAID-induced lesions in the small intestine in dogs. (Reprinted from Z. A. Radi and N. K. Khan, Effects of cyclooxygenase inhibition on the gastrointestinal tract, *Experimental and Toxicologic Pathology*, 58, pp. 163–173. Copyright © 2006, with permission from Elsevier.)

does of 10 or 20 mg/kg per day and GI effects were evaluated. Gastroduodenal ulcers and lesions occurred with indomethacin treatment at both doses. Additionally, indometacin produced focal ulcers in the cecum. The mucosal concentrations of indometacin in the gastric and intestinal mucosa correlated with mucosal injury (Rainsford et al., 2003). A 4-week toxicity study of indomethacin was conducted in nonhuman primates (marmoset) in which indomethacin was administered by oral route at dose levels of 2, 6, and 12 mg/kg per day. All animals given the daily 12-mg/kg dose and one animal given 6 mg/kg per day died during the dosing period and within 20 days. At 12 mg/kg per day, indomethacin induced severe GI toxicity, characterized by hemorrhage, ulcers, and necrosis with peritonitis (Oberto et al., 1990).

Diclofenac (Cataflam, Voltarin) has a rapid absorption, a short-half life of approximately 2 h, and is metabolized in the liver by CYP2C in humans. It is the most widely used NSAID in the world to treat RA, OA, and ankylosing spondylitis. The GI adverse effects of twice-daily administration of 75 mg of diclofenac were evaluated in one of the largest and longest individual-outcome randomized double-blind clinical studies of NSAID use in RA and OA patients. A total of 23,504 patients were randomized with mean treatment duration from 19.4 to 20.8 months (Combe et al., 2009). Significantly higher upper GI events (perforation, bleeding, obstruction, and ulcer) occurred with diclofenac than with 90- or 60-mg once-daily administration of etoricoxib (Combe et al., 2009). In rats, diclofenac acute (5 h) oral administration at 3.5, 7, and 15 mg/kg caused gastric ulcers. In rats treated with diclofenac at 15 mg/kg, pathological changes included longitudinal and diffuse gastric ulcers, particularly along the mucosal pleats, and thinning and inflammation of the intestinal wall with poor elasticity (Conforti et al., 1993).

Sulindac (Clinoril) is a prodrug whose anti-inflammatory activity (used to treat rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute gouty arthritis) resides in its sulfide metabolite. Sulindac is available in 200-mg tablets and undergoes two major biotransformations after oral administration. It is oxidized to the sulfone and then reversibly reduced to the sulfide. The sulfide is formed largely by the action of gut microflora on sulindac excreted in the bile. The half-life of the active sulfide is approximately 18 h. Sulindac can cause serious adverse GI events in humans, including inflammation, bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine (according to a sulindac package insert).

Nabumetone (Relfen) exerts its pharmacological effects via its metabolite 6-methoxy-2-naphthylacetic acid (6-MNA) and is used to treat RA and OA. 6-MNA is not a biliary secretion and is inactivated in the liver, then conjugated before excretion. Because it is a nonacidic, prodrug formulation, fewer GI events were observed after nabumetone treatment than after treatment with other NSAIDs. GI events in humans included perforations, ulcers and bleeding, nausea, abdominal pain, and dyspepsia (Bannwarth, 2008). No gastric damage was observed in a 3-day study in rats in which nabumetone was orally dosed at 79 mg/kg and 6-MNA was given IV at 34 mg/kg (Melarange et al., 1992). The GI tolerability and pathology of nabumetone and etodolac were evaluated in an extensive nonclinical acute and chronic safety study (Spangler, 1993). In a single-dose study, etodolac caused a significant increase in both gastric and intestinal damage at 6, 24, 48, and 144 h after dosing. In contrast, no significant GI damage was noted with nabumetone. In the 28-day study, a significant increase in GI damage was noted with etodolac, but not with nabumetone, despite the higher dose employed [the nabumetone dose was five times the ID_{25} (the dose that reduces inflammation by 25% in 50% of animals)] (Spangler, 1993).

Effects of Aminonicotinic Acid Derivative ns-NSAIDs on the GI Tract

Although not approved for use in cats, dogs, or food animals, flunixin meglumine (Banamine), an aminonicotinic acid derivative, is approved for use in nonhuman

primates and horses to control pain, colic, and endotoxic shock (Moore et al., 1981; Lees and Higgins, 1985; Kopcha and Ahl, 1989; Kallings et al., 1999). Renal excretion contributes significantly to flunixin meglumine in horses (Lees and Higgins, 1985). Flunixin meglumine can be administered to horses by IV, intramuscular, or oral routes at the recommended therapeutic dose of 1.1 mg/kg once a day for up to 5 days. Flunixin meglumine GI toxicity at recommended doses appears to be rare. Oral administration at three times the recommended dose for 10 days failed to elicit toxicity (Lees and Higgins, 1985). However, GI ulceration and erosion occurred in horses dosed 1.1 mg/kg IV every 8 h for 12 days (MacAllister et al., 1993). Tissue specimens were obtained from ischemic-injured and nonischemic jejunum immediately after ischemia and 18 h after recovery from ischemia. The investigators found that ischemic-injured tissue from horses treated with flunixin meglumine had significantly lower transepithelial electric resistance and retarded recovery of the jejunal mucosa barrier after 18 h of reperfusion (Campbell and Blikslager, 2000; Tomlinson et al., 2004). Additionally, this ns-NSAID was linked with GI ulceration and diarrhea in horses and dogs (Traub-Dargatz et al., 1988; Carrick et al., 1989; Vonderhaar and Salisbury, 1993; Luna et al., 2007). Flunixin meglumine has a significantly longer $t_{1/2}$ in cows (approximately 8 h) compared with horses (approximately 2 h) or dogs (approximately 4 h) and is used to treat bovine pneumonia at a dose of 2 mg/kg once a day for 3 to 5 days, as well as acute mastitis. No effects on the GI tract were noted when flunixin meglumin was given experimentally to calves (Kopcha and Ahl, 1989). In dogs, flunixin at 1 mg/kg for 3 days with 4-day intervals resulted in a significantly longer bleeding time and gastric lesions (Luna et al., 2007).

Effects of Pyrazolone Derivative ns-NSAIDs on the GI Tract

Phenylbutazone (Butazolidin), a pyrazolone derivative, is a widely studied pyrazolone ns-NSAID approved for use in dogs and horses to treat OA, osteoporotic conditions, and laminitis and studied experimentally in rats, cats, and food animals. Phenylbutazone metabolite is oxyphenybutazone. When used in dogs, phenylbutazone was less toxic to this species than to humans but induced blood dyscrasia and GI injury (Watson et al., 1980; Conlon, 1988; Johnston and Budsberg, 1997). In horses, phenylbutazone has a $t_{1/2}$ that ranges from 3 to 10 h and has a narrow therapeutic index that may be related to lower plasma protein binding (Tobin et al., 1986). Absorption of phenylbutazone from the GI is influenced by the dose administered and the relationship of dosing to feeding. Access to hay can delay the time of peak plasma concentration to 18 h or longer (Tobin et al., 1986). GI-associated toxicity in hoses includes gastric ulcers and erosions, edema of the small intestine, mucosal atrophy, duodenal erosions, erosions and ulcers of the large colon, and ulcerative colitis (Mackay et al., 1983; Traub et al., 1983; Collins and Tyler, 1985; Karcher et al., 1990; Meschter et al., 1990a,b). Phenylutazone resulted in more severe GI toxicity in horses than did ketoprofen and flunixin meglumine, causing edema in the small intestine, erosions and ulcerations in the large intestine, and gastric ulceration at a dose of 4.4 mg/kg IV every 8 h for 12 days (MacAllister et al., 1993). In addition, hypoproteinemia and hypoalbuminemia secondary to

protein-losing enteropathy was seen in these horses. A 10-mg/kg dose of phenylbutazone once daily for 14 days is considered toxic and caused weight loss, diarrhea, and GI erosions and ulcerations (MacAllister, 1983). In laboratory animals such as dogs and rats, phenylbutazone caused GI pathology of blood or discolored stools and intestinal ulceration and/or perforation. In dogs, daily oral dosing at 200 mg/kg per day (32.3-fold multiples of human dose) for three months caused GI lesions. In rats, repeated oral dosing at 50, 100, and 200 mg/kg per day (8.1-, 16.1-, and 32.3-fold multiples of human dose) for six months caused GI lesions (Hallesy et al., 1973).

In ruminants, phenylbutazone is used to control arthritis and laminitis and is absorbed slowly following oral administration and cleared more slowly than that in horses and dogs. Although it protected calves against local dermal inflammation and systemic shock, it partially blocked rumen stasis in goats (Van Miert et al., 1977; Eyre et al., 1981). In rats, it caused gastric mucosal ulceration, bleeding, and hemorrhage and small intestine perforation and adhesions (Shriver et al., 1977; Mersereau and Hinchey, 1981; Takeuchi et al., 2004). These lesions are attributed to increased gastric contractions induced by the drug (Mersereau and Hinchey, 1981).

Effects of Salicylic Acid Derivative ns-NSAIDs on the GI Tract

Acetylsalicylic acid (aspirin) is still used widely due to its analgesic, antipyretic, and anti-inflammatory properties. Some salicylates, such as sulfasalazine, olsalazine, and mesalamine, are used to reduce inflammation associated with inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis [UC] (Radi et al., 2011). These IBD drugs cause splitting of the diazo bond by colonic bacteria to give sulfapyridine and 5-aminosalicylic acid (5-ASA), which is considered to be the active moiety that is delivered to the GI mucosa (Robinson, 1989). Emerging data suggest that 5-ASA treatment reverses an imbalance between the angiogenic factor VEGF and the antiangiogenic factors endostatin and angiostatin in an experimental UC rat model (Deng et al., 2009). The authors conclude that the effect of 5-ASA in UC may be caused by the down-regulation of expression of endostatin and angiostatin by modulation of matrix metalloproteinases-2 (MMP2) and MMP9 via inhibition of TNF α (Deng et al., 2009). Acetylsalicylic acid is rapidly absorbed mostly from the upper small intestine and undergoes rapid metabolism to the hydrolyzed active product, salicylic acid. Acetylsalicylic acid is the only salicylate that irreversibly inhibits cyclooxygenase by covalent acetylation of the enzyme. Salicylic acid is eliminated by hepatic conjugation with glucuronide and glycine and by renal excretion through glomerular filtration. The safety margin of aspirin is generally wide. The elimination half-life of salicylate varies significantly across species. The $t_{1/2}$ in cats is 27 to 45 h, 4.5 to 8.5 h in dogs, 1 h in horses, and 0.5 h in cows. Therefore, aspirin dosages range from 10 to 20 mg/kg orally every 2 to 3 days in cats, 10 to 20 mg/kg orally every 12 h in dogs, and 100 mg/kg orally every 12 h in cows (Langston and Clarke, 2002). Comparison of NSAID glucuronidation between several species indicated that it was most potent in monkeys, dogs, and humans. Cats were efficient in that respect because cats tend to be deficient in some

glucuronyl transferases enzymes that are important for glucuronidation (Magdalou et al., 1990). As a result, drugs that are excreted as glucuronide conjugates in other species, such as aspirin and paracetamol (acetaminophen), may have a prolonged half-life in cats, therefore increasing the risk of toxicity due to drug accumulation. Oral bioavailability of aspirin may vary due to differences in stomach content and pH (Conlon, 1988). A rise in pH increases the solubility of salicylates, and salicylate excretion depends on urinary pH; therefore, the short $t_{1/2}$ in horses is related to the basic urinary pH. Acetylsalicylic acid is poorly absorbed from the GI tract of horses after oral administration and disappears rapidly from the plasma. Although not approved for use in small animals, aspirin is most commonly used in dogs, but with associated GI complications, including mucosal erosions and hemorrhage in the pyloric antrum, cardia, and lesser curvature of the stomach (Conlon, 1988). These findings are not unusual, considering that aspirin and sodium salicylate are readily absorbed from the stomach and intestine of dogs and cats. Aspirin is rapidly deacetylated to salicylate, which is toxic to cells, affects mucosal barrier function, reduces cytosolic adenosine triphosphate, stimulates sodium transport and permeability, and increases proton dissipation from surface epithelial cells, resulting in microvascular damage, inflammation, hemorrhage, and gastric ulceration (Kauffman, 1989). In humans, aspirin use is associated with upper GI bleeding related to gastric hemorrhage and erosions distributed throughout the antrum of the stomach, especially more proximate to the body of the stomach with GI clinical manifestations of stomach upset, nausea, constipation, and diarrhea (Cryer, 2002).

Aspirin is available in a variety of preparations, such as plain, buffered, time-release, and enteric-coated. A potential strategy to combat the adverse GI effects from aspirin is administration of buffered or enteric-coated aspirin, which may prove less irritating to the dog stomach (Kauffman, 1989). Doses of 25 mg/kg of plain aspirin given at 8-h intervals for seven treatments resulted in gastric mucosal erosions in dogs, whereas there was minimal damage in dogs receiving buffered and enteric-coated preparations (Lipowitz et al., 1986). Misoprostol, a synthetic PGE₁ analog, has also been effective at decreasing endoscopically detectable mucosal gastric lesions (submucosal hemorrhage, erosion, or ulceration) in dogs given aspirin (Gullikson et al., 1987; Murtaugh et al., 1993; Johnston et al., 1995). In a dog repeat oral dosing study of aspirin at 60 mg/kg per day (onefold multiple of human dose) for three months, GI pathology of bloody or discolored stools, intestinal ulceration and/or perforation was seen (Hallesy et al., 1973). Aspirin given at 300 and 600 mg/kg per day for 4 days to Lewis rats with adjuvant-induced arthritis caused gastric mucosal bleeding and submucosal hemorrhage, which was seen at various time points of 24, 48, 72, and 96 h postdosing at necropsy (Shriver et al., 1975; Shriver et al., 1977). There are differences in the susceptibility of normal and arthritic rats to the gastric lesion-inducing properties of aspirin, with arthritic rats being more sensitive than normal rats. Oral administration of aspirin at doses of 10, 20 and 40 mg/kg caused dose-related increases in both the percentage of rats with gastric lesions and the severity of gastric lesion formation in both arthritic and nonarthritic rats. However, arthritic rats were less able to cope with the aspirin-induced insult to the gastric mucosal barrier (Katz et al., 1987).

Effects of Anthranilic Acid Derivative ns-NSAIDs on the GI Tract

Anthranilic acid derivatives include meclofenamate and mefenamic acid (Table 1-3). Meclofenamate (Ponstel, Arquel, Meclofen) is highly protein bound, metabolized by the liver, excreted in the urine and feces, and indicated to treat RA and OA in humans and musculoskeletal pain and inflammation in veterinary medicine (horses, dogs, cows). In a metaanalysis clinical study of dyspepsia, increased risk of dyspepsia was observed in meclofenamate users and NSAIDs (indomethacin, piroxicam) (Ofman et al., 2003). Dyspepsia in this study included any outcome terms, such as epigastric or upper abdominal pain/discomfort, but did not include nausea, vomiting, or heartburn. In humans, however, the most common GI event with meclofenamate is diarrhea. In cattle, oral administration of meclofenamic acid results in a biphasic pattern of absorption. Peak plasma concentration occurs at approximately 30 min and this is followed by a second peak at 4 to 6 h after dosing. The second peak is presumed to be due to enterohepatic recirculation (Aitken and Sanford, 1975). In horses, meclofenamic acid is absorbed rapidly and is effective in treating acute and chronic laminitis. It has a narrow therapeutic window and the onset of action is slow, requiring 2 to 4 days of dosing for clinical efficacy (Lees and Higgins, 1985). In rats, repeated oral dosing of mefenamic acid at 50 and 100 mg/kg per day (3.3- and 6.55-fold multiples of human dose) for 18 months caused GI pathology of bloody or discolored stool and intestinal ulceration and/or perforation (Hallesy et al., 1973). In nonhuman primates, repeated oral dosing of mefenamic acid at 400 and 600 mg/kg per day (26- and 39-fold multiples of human dose) for two years caused GI pathology similar to that described above in rats (Hallesy et al., 1973).

EFFECTS OF COX-1 INHIBITORS ON THE GI TRACT

COX-1 deficiency or inhibition is compatible with normal small intestinal integrity (Sigthorsson et al., 2002). COX-1 knockout mice do not spontaneously develop GI lesions, demonstrating that the absence of COX-1 alone is not sufficient to induce GI pathology (Langenbach et al., 1995). COX-1-deficient (COX-1^{-/-}) mice are normal except for a decrease in intestinal PGE₂ levels (Sigthorsson et al., 2002). COX-1 inhibition alone does not cause GI injury. A selective COX-1 inhibitor (SC-560) did not cause intestinal damage in rats (Tanaka et al., 2002a). In an in vitro study of small intestine motility, SC-560 was devoid of significant effects on horse ileal motility (Menozzi et al., 2009). Another in vitro study utilized a pig ileal intestine ischemia model and found that exposure to SC-560 recovered injured tissue to control levels as assessed by transepithelial electrical resistance (Blikslager et al., 2002). The effects of SC-560, rofecoxib, and indomethacin on the healing of colon lesions induced by dextran sulfate sodium (DSS) in the rat were investigated. The investigators found that daily administration of indomethacin and rofecoxib significantly delayed the healing of colitis, with deleterious influences on histological restitution as well as mucosal inflammation, whereas SC-560 had

no effect (Tsubouchi et al., 2006). Okayama et al. (2001) found that SC-560 significantly worsened the severity of colonic damage in DSS-induced colitis in rats. Another study in a DSS-induced colitis mouse model found that rofecoxib ameliorated severe colitis and reduced the degree of inflammation by reducing neutrophil infiltration and IL-1 β levels (Martin et al., 2005). In healthy rats, neither the s-NSAID rofecoxib nor the COX-1 inhibitor SC-560 when given alone at 20 mg/kg induced gastric mucosal injury. However, when rats received concurrent treatment with both SC-560 and rofecoxib, severe gastric lesions developed (Gretzer et al., 2001). Recent data suggest that COX-1 inhibition via SC-560, but not COX-2-derived PGE₂ synthesis, is involved in augmentation of NSAID-induced gastric acid secretion in isolated rabbit stomach parietal cells by enhancing expression and activation of the proton pump (Nandi et al., 2009).

Studies in animal models suggest that inhibition of COX-1 and COX-2 is required for induction of gastric ulcerogenic action of ns-NSAIDs (Wallace et al., 2000; Tanaka et al., 2001, 2002a,b; Takeuchi et al., 2004). It is thought that NSAID-induced ulcerogenesis, at least in rats, is dependent on the amount of gastric acid secretion derived from increased proton pump expression and requires inhibition of both COX-1 and COX-2 (Zinkievich et al., 2010). For example, in an experimental mouse model, small intestinal ulcers were observed when celecoxib and SC-560 were administered concurrently, but no GI damage was observed when either compound was administered independently (Sigthorsson et al., 2002). The importance of COX-1 and COX-2 simultaneous inhibition to cause GI effects is further supported by findings by Wallace et al. and Tanaka et al. Wallace et al. (2001) reported that COX-1 inhibition in rats reduced gastric mucosal blood flow but did not increase leukocyte adherence to the mesenteric vessel wall. On the other hand, COX-2 inhibition increased leukocyte adherence but did not reduce gastric mucosal blood flow (Tanaka et al., 2001). Thus, in the normal gastric mucosa, at least in rats, increased leukocyte adherence and vasoconstriction act in concert to facilitate gastric mucosal damage and lesions develop only when mucosal microcirculation and leukocyte function are impaired simultaneously. Furthermore, no spontaneous GI lesions occurred in COX-1-knockout mice, although gastric PE₂ levels were <1% of those in wild-type animals (Langenbach et al., 1995). Similarly, no GI pathology was found in COX-2-deficient mice (Morham et al., 1995).

EFFECTS OF COX-2 S-NSAIDS ON THE GI TRACT

Several COX-2 s-NSAIDs are approved for use in human and veterinary medicine (Table 1-4). Numerous nonclinical studies have demonstrated and supported the reduced GI events of COX-2 s-NSAIDs. In rats, rofecoxib did not cause damage to the stomach or small intestine (Yokota et al., 2005). When administered either orally or subcutaneously in rats, rofecoxib did not produce pathological changes in the GI mucosa, which showed normal histology (Laudanno et al., 2001). Neither rofecoxib nor celecoxib (Celebrex) caused gastric damage in normal rats after oral administration; however, both drugs caused hemorrhagic gastric lesions in arthritic rats (Kato et al., 2002). However, another study investigated the effects of celecoxib

TABLE 1-4 Major COX-2 Selective NSAIDs

Generic name	Trade name
Rofecoxib	Vioxx
Celecoxib	Celebrex
Valdecoxib	Bextra
Lumiracoxib	Prexige
Etoricoxib	Arcoxia
Deracoxib	Deramaxx
Parecoxib	Dynastat
Firocoxib	Previcox
Robenacoxib	Onsior
Meloxicam	Mobic, Metacam
Mavacoxib	Trocoxil

and rofecoxib in an experimentally induced colitis rat model. Colitis was induced by intrarectal instillation of acetic acid, which caused hemorrhagic diarrhea and weight loss. Oral administration of celecoxib at 5 mg/kg or rofecoxib at 2.5 mg/kg given twice daily reduced the degree of hemorrhagic diarrhea and the weight loss and significantly reduced the degree of colonic injury (El-Medany et al., 2005).

Clinical studies have shown that unlike ns-NSAIDs (e.g., etodolac, naproxen, ibuprofen), rofecoxib (Vioxx) did not inhibit PG synthesis or cause GI mucosal injury, even at supratherapeutic doses (Laine et al., 1995, 1999; Hawkey et al., 2000; Wight et al., 2001). There was no difference in ulceration rates of rofecoxib-treated patients as compared with a placebo and a fourfold lower depression of PG synthesis than in ibuprofen-treated patients (Laine et al., 1999; Hawkey et al., 2000). The Vioxx Gastrointestinal Outcome Research Trial (VIGOR) was a large (conducted in 301 centers in 22 countries) 13-month placebo-controlled double-blind study that compared twice the recommended dose of rofecoxib (50 mg daily) with the most common dose of naproxen (1000 mg daily) in 8076 RA patients (Bombardier et al., 2000). The primary endpoint was symptomatic ulcers, including clinical upper GI events of perforation, obstruction, and bleeding. The secondary endpoint was complicated upper GI events (perforation, obstruction, and major bleeding, resulting in a drop of 2 g or more in hemoglobin, transfusion, or hypotension). The RA patient population of VIGOR was selected because RA patients use NSAIDs chronically and have a substantially higher risk of NSAID-related GI events than do patients with OA. Rofecoxib significantly decreased the incidence of all GI endpoints studied in VIGOR. The VIGOR study showed a 54% reduction in clinical ulcers and a 57% reduction in complicated upper GI events with rofecoxib as compared with naproxen (Bombardier et al., 2000). The rate of discontinuation for any GI events (including clinical endpoints) was significantly lower in the rofecoxib group than in the naproxen group (Bombardier et al., 2000). A trial of the assessment of differences between Vioxx and naproxen to ascertain gastrointestinal tolerability and effectiveness (ADVANTAGE) was a 12-week

double-blind randomized prospective trial in 5597 patients with OA in the United States and Sweden who were randomized to receive rofecoxib (25 mg daily) or naproxen (500 mg twice daily). Patients using low-dose aspirin (<81 mg/day) were included in the trial. The primary endpoint of ADVANTAGE was GI tolerability as defined by the incidence of discontinuations due to GI adverse events. The secondary endpoint was use of concomitant medication to treat GI symptoms. Most patients (71%) were women, and the mean age of study participants was 63 years. Twelve percent of patients used low-dose aspirin during the trial, and baseline characteristics of the treatment groups were similar. At the study end, a significantly lower rate of adverse GI event-related discontinuations had occurred with rofecoxib. Significantly fewer patients receiving rofecoxib required concomitant GI medications than patients receiving naproxen. Concomitant use of low-dose aspirin did not significantly affect relative rates of discontinuation due to adverse events, serious adverse events, or drug-related adverse events (Lisse et al., 2003).

Both nonclinical and clinical data show that COX-2 s-NSAIDs have a superior GI safety and improved GI tolerability profile to that of ns-NSAIDs. In dogs, no evidence of GI toxicity has been observed with celecoxib at supertherapeutic doses (Khan et al., 1997). In rats, celecoxib did not induce any damage to healthy stomachs or GI mucosa (Altinkaynak et al., 2003; Li et al., 2003), did not alter the gastric mucosal barrier (Coppelli et al., 2004), did not cause intestinal ulcers, and reduced the severity of experimental colitis (Cuzzocrea et al., 2001), but exacerbated inflammation-associated colonic injury in experimental colitis and damage induced in the stomach in a separate study (Khan et al., 1997; Zhang et al., 2004). In an experimental study in a rabbit model, the effects of valdecoxib on anastomotic healing 1 week following large bowel resection were investigated. Valdecoxib did not influence anastomotic healing or new vessel formation in the anastomotic region following large bowel resection (Neuss et al., 2009).

Similar to the nonclinical data, data from several clinical studies suggest that COX-2 s-NSAIDs have a superior GI safety profile to that of ns-NSAIDs. For example, CS-706, an s-NSAID, and naproxen were administered for 7 days to healthy men and women who did not have evidence of underlying GI lesions, and posttreatment upper GI endoscopy was conducted to assess and compare the development of GI petechiae, erosions, and ulcers. The extent of upper GI mucosal injury with CS-706 was statistically and significantly less than that for naproxen (Moberly et al., 2007). Another study compared the effects of valdecoxib (Bextra) and naproxen, administered for 6.5 days, on the upper GI mucosa of healthy older subjects (aged 65 to 75 years) as assessed by GI endoscopy. Valdecoxib was associated with a significantly lower rate of gastroduodenal, gastric, and duodenal ulcers than that of naproxen (Goldstein et al., 2006). In a 26-week clinical trial, the incidence of GI ulcers in patients receiving the COX-2 s-NSAID valdecoxib was significantly lower than in those receiving diclofenac. Additionally, valdecoxib was also associated with significantly improved GI tolerability than that with diclofenac (Pavelka et al., 2003). The incidence of upper GI bleeding and dyspeptic GI adverse experiences in patients with osteoarthritis was significantly lower with rofecoxib than with ns-NSAIDs (e.g., diclofenac, ibuprofen, nabumetone) (Langman

et al., 1999). The Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET) study compared the GI safety of lumiracoxib (Prexige) with ibuprofen and naproxen. Lumiracoxib in this TARGET study showed a three- to fourfold reduction in ulcer complications (Schnitzer et al., 2004). A large body of data has been published comparing the GI safety of COX-2 s-NSAIDs, celecoxib, and varied ns-NSAIDs. The Celecoxib Long-Term Arthritis Safety Study (CLASS) was a double-blind randomized controlled trial carried out in 7968 patients from 386 centers in the United States and Canada that compared a celecoxib dose of 400 mg twice daily (which is two- and fourfold the maximum dosage for RA and OA, respectively) with two ns-NSAIDs (diclofenac at 75 mg twice daily or ibuprofen at 800 mg three times daily) (Silverstein et al., 2000). The primary endpoint in CLASS was the incidence of ulcer complications (ulcer perforation, gastric outlet obstruction, or upper GI bleeding). The secondary endpoint was complicated and symptomatic ulcer events. Celecoxib in this CLASS study was associated with a lower combined incidence of symptomatic ulcers and ulcer complications than was ibuprofen or diclofenac (Silverstein et al., 2000). When compared with naproxen, celecoxib-treated patients also had lower rates of gastric, duodenal, and gastroduodenal ulcers (Goldstein et al., 2001). The Successive Celecoxib Efficacy and Safety Study (SUCCESS) was a 12-week double-blind randomized trial in 13,274 patients from 39 countries. The SUCCESS trial compared the incidence of upper GI hospitalizations in patients with OA taking celecoxib (200 or 400 mg daily), diclofenac (100 mg daily), or naproxen (1000 mg daily) (Singh et al., 2006). The rate of hospitalization was significantly lower in the celecoxib group. In addition, there were fewer ulcer complications in the celecoxib group than in the diclofenac or naproxen group, both in patients taking concomitant aspirin and in those not taking aspirin (Singh et al., 2006). In a separate study, video capsule endoscopy in healthy volunteers showed that celecoxib induced significantly less small bowel erosion than naproxen combined with omeprazole (Goldstein et al., 2007). In the Multinational Etoricoxib and Diclofenac Arthritis Long-Term (MEDAL) trial, the effects on GI outcome of etoricoxib (Arcoxia) and diclofenac were assessed. There were significantly fewer upper GI clinical events with etoricoxib than with diclofenac (Laine et al., 2007). Another clinical study demonstrated that several COX-2 s-NSAIDs (i.e., celecoxib, rofecoxib, valdecoxib, etoricoxib, lumiracoxib) offer greater upper GI safety and are better tolerated compared with ns-NSAIDs (Rostom et al., 2007). Upper GI mucosal effects were investigated for parecoxib (Dynastat) in a two-center double-blind randomized placebo-controlled study. Healthy subjects aged 65 to 75 years who were shown at baseline endoscopy to have no gastric or duodenal lesions received either 40 mg of parecoxib sodium IV twice daily for 7 days or 15 mg of ketorolac IV once daily for 5 days. No gastric or duodenal ulcers occurred in any subjects receiving parecoxib sodium. On the other hand, 23% of the ketorolac subjects had at least one ulcer, 16% had gastric ulcers, and 6% had duodenal ulcers (Stoltz et al., 2002). In another multicenter randomized double-blind placebo-controlled design, 123 adults with endoscopically confirmed normal upper GI mucosa received parecoxib sodium 40 mg twice daily for 7 days or ketorolac 30 mg four times daily for 5 days. No subjects treated with parecoxib sodium or placebo developed GI ulcers. Additionally, parecoxib sodium was

comparable to placebo with respect to the combined incidence of erosions or ulcers. Thus, parecoxib sodium has a GI safety profile superior to that of ketorolac (Harris et al., 2004). The Meloxicam Large-Scale International Study Safety Assessment (MELISSA) trial was a large-scale double-blind randomized international trial conducted over 28 days in 9323 patients with symptomatic OA. Patients received either meloxicam 7.5 mg or diclofenac 100 mg, and significantly fewer adverse events were reported by patients receiving meloxicam than by those receiving diclofenac. Of the most common GI adverse events, there was significantly less dyspepsia, nausea and vomiting, abdominal pain, and diarrhea with meloxicam than with diclofenac. Thus, meloxicam has a significantly improved GI tolerability profile than that of diclofenac (Hawkey et al., 1998). Similarly, in another study called SELECT (the Safety and Efficacy Large-Scale Evaluation of COX-Inhibiting Therapies), 4320 patients with exacerbation of OA were treated with the recommended dose of meloxicam (7.5 mg) or piroxicam (20 mg) once daily for 28 days. There was a significantly lower incidence of GI adverse events in the meloxicam than in the piroxicam group (Dequeker et al., 1998).

Deracoxib (Deramaxx), a COX-2 s-NSAID approved for use in dogs, is indicated for the control of postoperative pain and inflammation associated with orthopedic surgery and osteoarthritis. Once absorbed, deracoxib protein binding is >90% and the half-life is 3 h. In a toxicology safety study, micronized deracoxib in gelatin capsules was administered once daily to healthy young dogs at doses of 10, 25, 50, and 100 mg/kg of body weight for up to 14 consecutive days. At the high doses of 25, 50, and 100 mg/kg, reduced body weight, vomiting, and melena occurred. Necropsy revealed gross GI lesions in dogs from all dose groups. The frequency and severity of the lesions increased with escalating doses. At 10 mg/kg, moderate diffuse congestion of gut-associated lymphoid tissues (GALT) and erosions or ulcers in the jejunum occurred. At the highest dose tested, 100 mg/kg, all dogs exhibited gastric ulcers and erosions or ulcerations of the small intestines (according to a package insert). In other 21-day and six-month toxicology studies in healthy dogs, deracoxib at lower doses of 2, 4, 6, 8, and 10 mg/kg per day did not cause abnormal GI findings as assessed by clinical observations or gross or histopathological examinations at any dose level tested (Roberts et al., 2009). Postapproval experience revealed GI events (i.e., vomiting, anorexia, diarrhea, melena, inappetence, hematemesis, hematochezia, weight loss, nausea, ulceration, perforation). However, it is not clear from this postapproval experience if deracoxib was used at the recommended doses or other NSAIDs or steroids were used. Deracoxib should only be used at approved dosages. In a retrospective study in dogs treated with deracoxib, it was found that 55% of dogs have received deracoxib at a dosage higher than that approved by the U.S. Food and Drug Administration for the particular indication being treated. In addition, it was found that 59% of dogs have received at least one other NSAID or a corticosteroid in close temporal association (within 24 h) with deracoxib administration (Lascelles et al., 2005a). Therefore, GI perforation has been observed in dogs that received deracoxib at a higher than approved dosage or had received at least one other ns-NSAID in close temporal association with deracoxib administration (Lascelles et al., 2005a). A randomized placebo-controlled trial compared gastroscopic findings in dogs given

aspirin (25 mg/kg) or deracoxib (1.5 mg/kg) for 28 days. The study found no significant differences in total scores between placebo and deracoxib-treated dogs on days 6, 14, and 28 and concluded that administration of deracoxib to healthy dogs resulted in significantly lower gastric lesion scores and fewer days of vomiting than with administration of aspirin (Sennello and Leib, 2006).

Another COX-2 s-NSAID, firocoxib (Previcox), has been proven clinically to control OA pain and inflammation in dogs (Pollmeier et al., 2006; Ryan et al., 2006). No adverse GI, hematological, or serum biochemical adverse effects were seen after oral daily administration of firocoxib for 29 days in healthy dogs (Steagall et al., 2007). In a large study with more than 1000 dogs with OA, a small withdrawal rate of 2.9% due to firocoxib GI-associated effects was observed, and no serious drug-related adverse events were reported (Ryan et al., 2006). The overall clinical efficacy of firocoxib to treat OA in horses was comparable to the ns-NSAID phenylbutazone (Doucet et al., 2008). In a study in healthy dogs, the gastric and duodenal effects of COX-2 s-NSAIDs after oral administration were investigated. Each dog received deracoxib (2 mg/kg), firocoxib (5 mg/kg), or meloxicam (0.2 mg/kg) for 3 days with a 4-week interval between successive treatments. No significant differences were found among these COX-2 s-NSAIDs regarding endoscopic GI mucosal scores, histologic scores, or COX-1 or COX-2 protein expression (Wooten et al., 2009). The effects of firocoxib on ischemic-injured jejunum mucosal recovery in horses were compared to those of flunixin meglumine. Transepithelial resistance of ischemic-injured jejunum from horses treated with flunixin meglumine was significantly lower than in firocoxib-treated horses (Cook et al., 2009). In a study in dogs, the effects of firocoxib on healing of induced gastric body and pyloric lesions were examined. Dogs were treated with firocoxib [5 mg/kg orally (PO) every 24 h] or placebo for 7 days. Healing was evaluated on days 2, 4, and 7 of treatment by endoscopic lesion scoring. Eicosanoid concentrations in plasma and at the lesion margins were determined on days 2, 4, and 7. The firocoxib group had larger pyloric lesions than the placebo, but mucosal PG production did not differ significantly from that with placebo (Goodman et al., 2009). In a blinded randomized crossover study design, cats were treated with firocoxib (1 mg/kg PO per day) and meloxicam (0.05 mg/kg PO per day) for 8 days. Blood samples and gastric and duodenal mucosal biopsy specimens were collected on days 0 (baseline; immediately before treatment), 3, and 8 of each treatment period. Firocoxib and meloxicam administration resulted in a lower plasma PGE₂ concentration than at baseline on days 3 and 8 of administration. Neither firocoxib nor meloxicam administration altered pyloric or duodenal PGE₁ synthesis (Goodman et al., 2010).

A recent COX-2 s-NSAID is robenacoxib (Onsior), which is prescribed to relieve pain and inflammation in cats and dogs. It contains four fluorine atoms and a carboxylic acid group and is chemically related to diclofenac and lumiracoxib. However, in contrast to most COX-2 s-NSAIDs, robenacoxib lacks a sulfur-containing group and is therefore considered to be chemically distinct from both the sulfone-containing rofecoxib and firocoxib class and the sulfonamide-containing celecoxib and deracoxib class (King et al., 2009). Significantly less gastric ulceration and intestinal permeability were noted in rats treated with robenacoxib

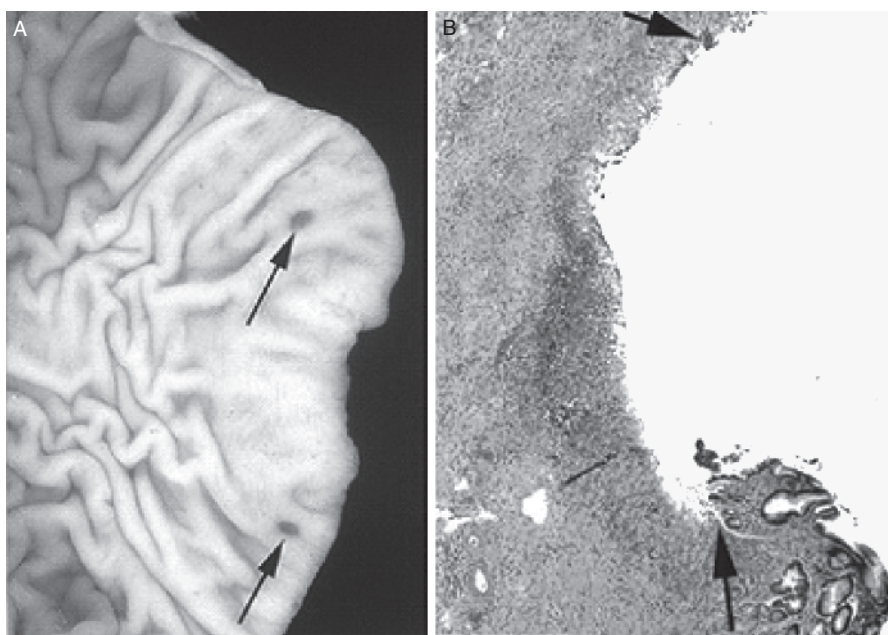


FIGURE 1-4 Meloxicam-induced gross (A) and microscopic (B) ulceration (between arrows) involving the mucosa in the pyloric region of the stomach of a dog. (Reprinted from Z. A. Radi and N. K. Khan, Effects of cyclooxygenase inhibition on the gastrointestinal tract, *Experimental and Toxicologic Pathology*, 58, pp. 163–173. Copyright © 2006, with permission from Elsevier.)

than in those treated with diclofenac (King et al., 2009). Another recent COX-2 s-NSAID is mavacoxib (Trocoxil), which is intended for the treatment of pain and inflammation associated with degenerative joint disease in dogs. No published GI safety data are currently available on this drug.

Other drugs previously compared to ns-NSAIDs for GI-related toxicity effects include meloxicam, L-745337, nimesulide, NS-398, and SC-58125. Meloxicam (Metacam, Mobic) is approved for use in dogs and cats and produced mild to moderate gastroduodenal lesions in dogs (Fig. 1-4) (Forsyth et al., 1998; Radi and Khan, 2006b; Radi, 2009); L-745,337 [5-methanesulfonamide-6-(2,4-difluorothiophenyl)-1-indanone] caused intestinal perforation in rats (Schmassmann et al., 1998); nimesulide did not cause any GI inflammation or ulcers in rats at excessive doses (Sigthorsson et al., 1998; Kataoka et al., 2000), prevented indomethacin-induced gastric ulcers in rats (Karmeli et al., 2000), and significantly decreased the extent of colitis induced by acetic acid in rats (Karmeli et al., 2000).

NS-398 produced little to no gastric ulceration in rats (Futaki et al., 1993; Masferrer et al., 1994) but had no beneficial effect on experimental colitis, whereas indomethacin did (Masferrer et al., 1994; Lesch et al., 1999). Similar to NS-398, SC-58125 yielded no GIT-related toxicity or beneficial effect in experimental colitis

in rats (Seibert et al., 1994; Lesch et al., 1999). The COX-2 s-NSAID NS-398 delayed healing after acid-induced gastric injury in rats (Sun et al., 2000).

PATHOPHYSIOLOGY AND MECHANISMS OF NSAID-ASSOCIATED GI TOXICITY

The GI tract is the site of entry into the body of orally administered drugs. Due to its high metabolic and mitotic rates, the GI system is susceptible to xenobiotics toxicity. Clinical signs of NSAID-associated GI system toxicoses (i.e., vomiting and diarrhea) are generally manifestations of the body's attempt to eliminate or reduce exposure to toxicants. The medullary emetic (vomiting) center can be stimulated by stomach irritation and distension. Bright red or dark-colored vomitus (hematemesis) indicates retention of blood in the stomach due to gastric ulceration. GI bleeding can be either in the upper GI tract (occurring in the stomach or duodenum) or in the lower GI tract (occurring in jejunum, ileum, colon, or rectum). The signs of GI bleeding include hematemesis, melena, hematochezia, or occult bleeding. Hematemesis indicates bloody vomitus that is either fresh, bright red blood, or dark, digested blood. Melena is black, tarry, foul-smelling stool caused by digestion of blood in the GI system. Hematochezia indicates fresh, bright red blood passed from the rectum. Occult bleeding is seen when trace amounts of blood are detected in normal-appearing stools. An ulcer is a local defect or excavation of the GI mucosal surface that is produced by sloughing of necrotic tissues and extends into the submucosa or deeper (Figs. 1-2 to 1-4) (Radi and Khan, 2006b). One of the consequences of GI mucosa inflammatory necrosis is ulceration. Erosion is a local defect of the GI tract that is confined to the mucosa and does not penetrate the submucosa and muscularis mucosa. The prevalence of endoscopically detectable gastroduodenal erosions associated with NSAID use in humans ranges from 14 to 60%, whereas the incidence of ulcers is 14 to 31% (La Corte et al., 1999). It is estimated that the annual number of hospitalizations in the United States from NSAID-associated GI complications is at least 103,000. Also, the estimated cost of such hospitalization is \$15,000 to \$20,000 per hospitalization, and the annual direct costs of such complications exceed \$2 billion (Wolfe et al., 1999). Although NSAIDs are known to produce GI lesions in various animal species, the intraanatomical incidence of ns-NSAID-induced GI lesions in veterinary medicine is unknown, but the gross and histopathological changes in dogs treated experimentally with loxoprofen sodium were erosions and ulcerations that were limited to the pyloric gastric mucosa, jejunum, and ileum mucosa (Korytko et al., 2003). GI-associated toxicity seen with ns-NSAIDs may be attributed to direct nonspecific irritation or to various biochemical and pharmacological mechanisms linked to local COX inhibition (Hawkey and Skelly, 2002; Radi and Khan, 2006b; Radi, 2009) (Fig. 1-1). The dual-insult hypothesis by Schoen and Vender (1989) suggests that NSAIDs exert a direct toxic effect on the GI mucosa and indirect effects through active hepatic metabolites and decreases in mucosal PGs. Hepatic metabolites are excreted into the bile and subsequently into the duodenum, where they cause mucosal damage to the stomach by duodenogastric reflux and mucosal

damage to the small intestine by antegrade passage through the GI tract. Detailed pathophysiological and potential mechanisms of NSAID-associated GI toxicity are discussed below.

Role of Cyclooxygenase Potency

Cyclooxygenase inhibitory potency is an important factor in NSAID GI toxicity. An indicator of the COX-1 versus COX-2 activity at therapeutic doses is the COX-1:COX-2 IC₅₀ ratio in human blood. The COX-1:COX-2 IC₅₀ ratio in human blood (the concentration of drug that inhibits 50% of the COX-1 activity in blood to that which inhibits COX-2 in the blood) is a good indicator of enzyme specificity; numbers greater than 1 are more COX-2 specific, and those below 1 are more COX-1 specific. There is wide variation between reports in the exact values, but the differences in COX selectivity of drugs tested have fairly good correlation between studies. Cryer and Feldman (1998) reported that the COX-1:COX-2 IC₅₀ ratio of aspirin was 0.32, whereas ibuprofen was 0.6, naproxen was 1.14, and NS-398 was 24. Another report by Chan et al. (1999) indicated that the COX-1:COX-2 IC₅₀ ratio in human blood for rofecoxib was 36, celecoxib was 6.6, and diclofenac was 0.4. As discussed previously, a lower incidence of adverse GI NSAID effects occurs more often with COX-2 s-NSAID. For example, substantial inhibition of platelet COX-1 will translate into an increased incidence of serious upper GI bleeding and complications (Patrino et al., 2001). Furthermore, the results of TARGET and VIGOR showed that ns-NSAIDs (naproxen and ibuprofen) cause more GI adverse events than do COX-2 s-NSAIDs (lumiracoxib and rofecoxib), which spare COX-1 activity at therapeutic dosing (Bombardier et al., 2000; Schnitzer et al., 2004).

Species Differences in NSAID-Associated Susceptibility to GI Injury

Although ns-NSAID-associated GI toxicity is prevalent throughout species, there are significant interspecies GI toxicity differences (Table 1-2) (Mahmud et al., 1996; Radi and Khan, 2006b). The degree and susceptibility of GI-associated toxicity can vary depending on the drug administered and the species tested (Radi and Khan, 2006b; Radi, 2009). For example, fenbufen has resulted in significantly fewer gastric lesions (hemorrhage and ulceration) in humans than have other ns-NSAIDs, such as naproxen and indomethacin (Lanza et al., 1983). Naproxen is well tolerated in mice, rabbits, nonhuman primates, and pigs, less well tolerated by rats, and poorly tolerated by dogs (Hallesy et al., 1973). The susceptibility of cats, dogs, and horses to the adverse effects of ns-NSAIDs is greater than that of humans (Mahmud et al., 1996). Dogs are considered a sensitive laboratory animal species for predicting the toxicity profile of NSAIDs. The no observed effect level (NOEL) dosages for GI toxicity in dogs are at or below clinical therapeutic dose levels for NSAIDs and are severalfold higher for COX-2 s-NSAIDs (Korytko et al., 2003). GI toxicity occurred at subtherapeutic ns-NSAID lloprofen exposures (Korytko et al., 2003). There are significant breed differences in susceptibility

to GI ulceration subsequent to ibuprofen exposure, with lower risk for the labrador breed and higher risk for the German shepherd breed (Poortinga and Hungerford, 1998). However, dogs tolerate ns-NSAIDs better than cats do. Since cats lack the glucuronyl transferase enzyme, they are delicately sensitive to the adverse effects of drugs that are glucuronidated before elimination (i.e., acetaminophen) (Court and Greenblatt, 1997). In addition, cats are at least twice as sensitive to ibuprofen as dogs are (Villar et al., 1998).

In addition, it is important not to extrapolate from studies performed in one species to another since various NSAID pharmacokinetics (PK) (a drug's absorption, distribution, metabolism, and elimination) are different across species (Lees et al., 2004). Some of the general PK properties of NSAIDs are (1) good bioavailability in monogastric species after oral dosing because of a medium to high level of lipid solubility, (2) dissolution in stomachs impaired by acidic pH, (3) possible delayed absorption by binding to digesta (e.g., horses), (4) a high degree of plasma protein binding of all drugs (except salicylate) in all species and small volume of distribution, (5) a low volume of distribution (some exceptions), and (6) marked species differences in clearance and terminal half-life (Lees et al., 2004). Such PK properties have implications in GI toxicity. For example, meloxicam has nearly 100% bioavailability after subcutaneous injection in cats, with an elimination half-life after a single dose estimated to be approximately 15 h. Therefore, the recommended dose of meloxicam (according to a package insert) in cats is 0.3 mg/kg, to be given subcutaneously only once, due to the narrow safety margin in cats. Repeated use of meloxicam in cats has been associated with diarrhea, vomiting, lethargy, and decreased appetite. Histopathological examination revealed gastrointestinal lesions ranging from GI mucosal inflammatory cell infiltration to erosions. In dogs, aspirin GI-associated side effects are dose- and preparation-related. A dose of 25 mg/kg (but not 10 mg/kg) of plain aspirin caused mucosal erosions in dogs, while no GI damage occurred in animals receiving buffered and enteric-coated preparations at similar doses of 25 mg/kg (Lipowitz et al., 1986).

GI Anatomical Differences

GI anatomical and physiological differences should be taken into consideration when NSAIDs are administered and potential GI toxicity is anticipated. For example, unlike humans, rats cannot vomit and are considered a nonvomiting species. This is due to a fold in the stomach that lies where the esophagus enters (Fox et al., 2002). Rats lack a gallbladder, and bile enters the duodenum continuously as it is made. Dogs, on the other hand, are very susceptible to vomiting. Gut blood flow varies across species: 1.5, 7.5, 111, 216, 125, and 1100 mL/min in the mouse, rat, rabbit, dog, monkey, and human, respectively (Davies and Morris, 1993). Gut volume also varies across species: 1.5, 11.3, 120, 480, 230, and 1650 mL in the mouse, rat, rabbit, dog, monkey, and human, respectively (Davies and Morris, 1993). Humans, rhesus monkeys, and dogs are monogastric, and their stomachs are entirely secretory, whereas the stomachs of rodents, pigs, and horses have a glandular secretory portion and nonglandular bacterial digestion portion. Compared to humans, the GI length is shorter and

the gastric emptying time is longer in dogs. Particle size can affect the rate of gastric emptying. The rate of gastric emptying in minipigs of nondigestable tablets (11 mm diameter) and granules (1 mm diameter) is slower than that in humans and dogs (Aoyagi et al., 1992). The rate of gastric emptying of both dosages in the dog tended to be faster than or similar to that in humans (Aoyagi et al., 1992). Food delayed gastric emptying in dogs, especially for tablets (Aoyagi et al., 1992). Such anatomical differences can affect the rate and extent of absorption of some NSAIDs. For example, in humans, enteric-coated aspirin was designed to reduce stomach irritation by delaying absorption until the drug reached the small intestine. In a study where dogs were administered enteric-coated aspirin, oral absorption was incomplete, gastric retention of tablets occurred, and partially digested tablets were found in the feces of some dogs (Nap et al., 1990). Ruminants have a forestomach (comprised of rumen, reticulum, and omasum) that is followed by a true stomach (abomasum). The rumen is highly permeable to volatile fatty acids released from carbohydrate metabolism and is also capable of active sodium and chloride absorption. Therefore, the large size of the rumen affects the response to oral toxicants, and initial exposure to toxicants may result in dilution and slowing of the absorption rate. Because the horse is a monogastric animal, the aspirin is absorbed rapidly following oral administration but is also removed rapidly, due to its short $t_{1/2}$. One might assume that the recommended 100-mg/kg dose of aspirin to a cow might be quite toxic; however, in this case the rumen acts as an “anatomical sustained-release device,” slowly releasing the aspirin into the intestine for subsequent absorption. This slow release helps avoid potential aspirin-induced GI toxicity but allows for the maintenance of therapeutic drug concentrations (Langston and Clarke, 2002).

Enterohepatic Recirculation and NSAID Toxicity

The movement of a drug absorbed into the blood from the small intestine lumen, then carried into the liver by the hepatic portal vein, biotransformed in the liver, excreted into the bile, then through the bile duct into the lumen of duodenum, and reabsorbed into the blood via intestinal vessels is referred to as enterohepatic recirculation. Enterohepatic recirculation allows recycling of metabolized and nonmetabolized compounds and plays a role in NSAID-associated GI toxicity. Administration of NSAIDs that are not subject to enterohepatic recirculation did not produce intestinal damage in rats (Reuter et al., 1997). Additionally, ligation of the common bile duct prevented the damage normally observed after NSAID administration (Wax et al., 1970). Toxicological consequences of enterohepatic recirculation include increased drug half-life in blood, prolonged exposure, and inhibition of conjugate exports. For example, ibuprofen is particularly ulcerogenic in dogs because it undergoes enterohepatic recirculation. Indomethacin and piroxicam undergo substantial enterohepatic recirculation. Indomethacin enterohepatic recirculation is most extensive in dogs and rats and least extensive in rabbits and humans. In the dog, indomethacin remains in the enterohepatic circulation, even though it is reabsorbed from the gut, until its elimination in the feces. Biliary excretion of indomethacin and its conjugate is extensive and rapid in dogs, but slow in rats (Yesair et al., 1970). In

humans and nonhuman primates, most NSAIDs undergo less enterohepatic recirculation than in the dog and rat (Beck et al., 1990). Dogs are less tolerant than rats or humans to ns-NSAIDs and more at risk for ns-NSAID-induced gastropathy (Elliott et al., 1988; Forsyth et al., 1998). Many NSAIDs, except aspirin and salicylic acid, undergo enterohepatic recirculation in rats (Beck et al., 1990). Nabumetone is formulated as a nonacidic prodrug that does not undergo enterohepatic recirculation to improve its GI safety profile.

Role of Xenobiotic Glucuronidation

Glucuronidation, catalyzed by the UDP-glucuronosyltransferase (UGT) enzyme, is a major elimination and biotransformation pathway of endogenous and exogenous xenobiotic compounds. NSAIDs are eliminated primarily through conjugation with polar sugar moieties to form glucuronides. The UGTs catalyze the conjugation of compounds that possess a nucleophilic acceptor group with glucuronic acid, a relatively bulky, hydrophilic moiety, whose carboxylic acid functional group is ionized at physiological pH, thus forming metabolites with significantly different chemical and biochemical properties that in most cases have significantly decreased affinity for receptors or enzymes responsible for the biological activity of the parent compound (Siraki et al., 2005). A major biotransformation pathway for carboxylated NSAID is glucuronidation, with the resulting production of reactive acyl glucuronides (Siraki et al., 2005). Therefore, glucuronidation appears to play a role in NSAID GI-associated toxicity. Cats have remarkably low levels of UGT; therefore, they have a low capacity for hepatic glucuronidation and are more sensitive than other species to NSAID-associated GI toxicities (Court and Greenblatt, 1997; Steagall et al., 2009). Adduction, or covalent binding, of a toxicant to target macromolecules is considered a molecular mechanism of cellular injury of toxicants that damage tissues. For example, adducts detected by immunohistochemistry in the small intestine of diclofenac-treated rats represent covalent bonding of some diclofenac entity with intestinal macromolecules (Atchison et al., 2000). In addition, adduct formation after reactive metabolite generation by NSAIDs is one possible explanation for the mechanism of gut-induced toxicity. Rats given diclofenac orally at doses ranging from 10 to 100 mg/kg led to dose-dependent formation of adducts and ulcers only in the small intestine and only in animals with intact enterohepatic recirculation. Adducts formed within enterocytes by 1 h translocated to the brush border, preceded intestinal ulceration, and were intense at sites of ulceration (Atchison et al., 2000).

A major prerequisite for the enterohepatic recirculation of an NSAID is its conjugation by in the liver, which allows for hepatobiliary excretion of these highly polar metabolites. As discussed previously, in some NSAIDs (i.e., aspirin, diclofenac, ibuprofen, and indomethacin) intestinal toxicity is dependent on biliary excretion and enterohepatic circulation (Beck et al., 1990). Many carboxylic acid NSAIDs form ester glucuronides that are readily transported across the hepatic canalicular membrane by multidrug carrier systems. For example, diclofenac glucuronide is transported into bile by the canalicular conjugate export pump, Mrp2, in the rat (Seitz et al., 1998). Hepatocanalicular conjugate export pump-deficient

(TR⁻) rats were used to selectively block diclofenac enterohepatic circulation without interrupting bile flow. TR⁻ rats were refractory to diclofenac given either intraperitoneally or perorally. However, transfer of bile containing diclofenac glucuronide significantly increased the extent of ulcer formation in both normal and TR⁻ rats. Moreover, induction of glucuronosyltransferase aggravated intestinal ulceration (Seitz and Boelsterli, 1998).

Aging and Stress and NSAID GI Effects

Age appears to be a contributing factor in NSAID-induced GI toxicity. The exact mechanism of this is not fully understood. It is thought that the aging gastric mucosa has impaired mucosal defense mechanisms, due to decreased mucus and bicarbonate secretion, reduced gastric emptying rate, reduced GI motility, reduced GI blood flow, increased expression on villi of enterocytes and the function of the P-glycoprotein multidrug efflux pump, and reduced GI PG production. Neonates have a poorly developed intestinal mucosa barrier that can permit absorption of various xenobiotics. Some studies in humans have demonstrated a decline in gastric and duodenal mucosal PG content with aging, associated with an increase in gastric acid secretion (Cryer et al., 1992; Goto et al., 1992). Similarly, studies in animals have yielded similar results (Uchida et al., 1990; Lee and Feldman, 1994). Uchida et al. (1990) have demonstrated a marked decrease in gastric mucosal PGI₂ level between 20 and 40 weeks of age and between 60 and 86 weeks of age in normal and ulcer-bearing rats. In another study, gastric mucosal PGs synthesis decreases with age in rats, and aged animals were more susceptible to aspirin-induced acute gastric mucosal injury (Lee and Feldman, 1994).

In a study in humans, Feldman and Cryer (1998) demonstrated that in healthy subjects with normal gastric histology, advancing age was associated with a significant decline in gastric bicarbonate secretion (HCO₃⁻), Na⁺, and nonparietal fluid secretion, resulting in an increase in gastric acidity, while no age-related changes in acid and parietal fluid secretion were noted. Furthermore, animal studies have shown that aging was associated with significantly lower gastric luminal pH and bicarbonate output in the rat stomach and that aging also blunted PG-mediated increases in gastric HCO₃⁻ secretion (Lee, 1996). Moreover, Kim et al. (1990) have shown in anaesthetized rats that although aging does not affect basal duodenal bicarbonate secretion, the duodenal bicarbonate response to a fixed load of luminal acid declines progressively with age. Finally, some NSAID clearance is altered with aging. Clearance of phenylbutazone is twice as fast in 3-year-old horses as in 8- to 10-year-old horses (Tobin et al., 1986).

A syndrome in humans called stress-related mucosal disease (SRMD) is common in critically ill patients and can result in significant morbidity. Histologically, there does not appear to be a significant inflammatory component to the gastric mucosa. The pathophysiology of this condition is multifactorial, but local mucosal ischemia and gastric acid play a critical role in disease pathogenesis. It is thought that gastric mucosal damage results from an imbalance between factors promoting mucosal injury and host defenses. A major mucosal defense factor is impaired

mucosal blood flow. Aggregative factors include acid, pepsin, intramucosal acidosis, reperfusion injury, and free-radical formation (Duerksen, 2003). There does not seem to be a selective impairment of PG production in patients with SRMD. Therefore, there is no evidence that NSAIDs increase the susceptibility of critically ill patients to stress-related GI damage (Duerksen, 2003).

Disruption of GI Physiological Mucosal Defense Mechanisms

The GI system is involved primarily in breaking down food, ultimately absorbed into the body. This digestive process involves several phases: ingestion of food; secretion of mucus, water, and enzymes; fragmentation; chemical and mechanical digestion of food particles; absorption of digested food; and elimination of waste products by defecation. Fragmentation and initial digestion take place in the stomach. The stomach has gastric glands that release or secrete mucus, hydrochloric acid (HCl), gastrin, somatostatin, acetylcholine, histamine, and pepsinogen. The small intestine is a major GI site of digestion and absorption of nutrients and electrolytes. The small intestine is divided into the duodenum, jejunum, and ileum. The process of digestion is initiated in the stomach by the actions of HCl and pepsin, which break down food particles. The mucosa of the stomach and proximal duodenum are constantly exposed to gastric acid that can damage living cells. Acid secretion is stimulated by acetylcholine neurotransmitter, gastrin hormone, and histamine. PGs can inhibit acid secretion. The gastroduodenal lumen also contains bile salts and enzymes such as pepsins, lipases, proteases, and peptidases (Johnson et al., 2006). Proton pump inhibitors (PPIs) significantly decrease NSAID-induced gastric and duodenal ulcers (Lazzaroni and Porro, 2009). PPIs are a group of drugs whose main action is pronounced and long-lasting reduction of gastric acid production.

The GI mucosa has several physiological defense mechanisms that form a mucus coating called a mucosal barrier to protect itself from the damaging effects of such degradative enzymes and acidic pH. Major physical and chemical gastric mucosa defense mechanisms include a hydrophobic mucus layer, regulated intercellular tight junctions, specialized plasma membrane ion permeability, epidermal growth factors, HCO_3^- secretion, high rate of mucosal blood flow, mucosal cell hydrophobicity, and rapid epithelial turnover (Johnson et al., 2006). In addition, the gastric and duodenal mucosa are rich in PGs, which play a protective role in the GI tract via adequate perfusion of the gastroduodenal mucosa, epithelial cell secretion of bicarbonate, secretion of mucus, and maintenance of a neutral mucosa pH (Elliott et al., 1996; Scheiman, 1996).

The mucus hydrophobic layer traps secreted bicarbonate to maintain a neutral gastric mucosal pH and forms a stable water-insoluble glycoprotein gel that acts as a lubricant to prevent mechanical damage. The mucus–bicarbonate layer protects the gastric mucosa from diffusion of free hydrogen ions from the gastric lumen back into the mucosal cells (i.e., back-diffusion) (Johnson et al., 2006). If the integrity of the GI barrier is disturbed, the rate of back-diffusion of gastric acid and pepsin increases, leading to inflammation and hemorrhage. Inflammatory cells such as neutrophils and mast cells become activated and release inflammatory

mediators such as histamine, leukotriens, free radicals, and proteolytic enzymes. These mediators lead subsequently to vasodilation, vasoconstriction, increased vascular permeability, and edema. Such events lead to GI mucosal ischemia, reduced mucus secretion, and reduced PG production (McConnico et al., 2008).

Inhibition of PG synthesis and abrogation of protective mechanisms lead to GI injury (Hawkey and Skelly, 2002). Misoprostol is a synthetic PGE₁ analog used to overcome NSAID-induced PG deficiency in the gastric mucosa. In the six-month randomized Misoprostol Ulcer Complication Outcomes Safety Assessment (MUCOSA) trial, the effects of concurrent administration of misoprostol on the occurrence of serious upper GI complications in patients with RA who were receiving NSAID were investigated (Agrawal and Aziz, 1998). The results of the MUCOSA study showed that misoprostol resulted in a statistically significant reduction in the incidence of serious NSAID-induced upper GI complications compared with placebo in patients with RA (Agrawal and Aziz, 1998). NSAIDs can also decrease mucosal resistance in the diseased state (Rainsford, 1982) or cause direct chemical damage to the GI mucosa (Johnston and Budsberg, 1997). When the GI mucosal integrity is compromised, a cascade of pathological events follows, leading to further mucosal barrier layer damage. COX inhibition is suggested to increase the susceptibility of the gastric mucosa to injury by inhibiting secretion of the cytoprotective mucus and bicarbonate and altering the physicochemical nature of mucus (Kauffman, 1989). Indomethacin and SC-560, but not rofecoxib, attenuated mucosal acidification in the rat stomach (Takeuchi et al., 2006). This study suggests that EP₁ receptors are essential for the increase in the secretion of HCO₃⁻ in response to mucosal acidification in the rat stomach. In EP₃ receptor–knockout mice, the HCO₃⁻ stimulatory action of PGE₂ was observed in the stomach, whereas such action was absent in the EP₁ receptor–knockout mice (Takeuchi et al., 1999). Therefore, it appears that PGE₂ receptor subtypes (EP₁ in the stomach and EP₃ in the duodenum) and COX-1 are key regulators of HCO₃⁻ secretion in response to gastroduodenal mucosal acidification.

GI Disequilibrium

All segments of the GI from duodenum to distal colon have mechanisms for both absorbing and secreting water and electrolytes. GI secretions play a significant role in digestion and maintenance of pH levels and acid–base balance. Disequilibrium in GI motility and secretion by NSAIDs can lead to diarrhea, dehydration, and/or systemic acidosis or alkalosis. Respiratory alkalosis may occur from stimulation of the respiratory center by phenylbutazone. Major mechanisms of diarrhea are osmosis, active secretion, exudation, and/or altered motility. The Na⁺ gradient is the driving force for amino acid, oligopeptide, and sugar absorption. There are differences in Na⁺ entry mechanisms, sites of HCO₃⁻ secretion, and sites of active K⁺ transport. HCO₃⁻ is absorbed in the jejunum (via Na⁺/H⁺ exchange) and secreted in the duodenum, ileum, and colon. Na⁺ crosses the small intestinal and colonic brush borders via Na⁺/H⁺ exchange and in the small intestine also by the Na⁺ organic solute cotransport mechanism. In the distal colon, luminal Na⁺ is also absorbed via an aldosterone-sensitive Na⁺ channel. The intestines neither dilute

nor concentrate their contents, the osmolarity of which, except in the duodenum and proximal jejunum shortly after eating, is the same as the plasma osmolarity. Osmotic diarrhea occurs when these organic solutes are absorbed, salt is absorbed with them, and water follows osmotically (i.e., transport from enterocyte to lateral intercellular space creates a local osmotic gradient that initiates water flow) (Field, 2003).

In osmotic diarrhea, agents released from inflammatory cells (PGs and leukotrienes, platelet-activating factor, histamine, serotonin) can stimulate active secretion. GI mucosal-induced inflammation interferes with GI homeostatic control. PGE₂ activates mast cells, causing histamine release, which stimulates smooth muscle activity and induces the secretion of mucus and electrolytes. Electrolytes create osmotic force for the influx of water into the lumen. Therefore, the combination of excessive fluids and increased GI motility causes diarrhea. The pathogenic importance of intestinal hypermotility in the intestinal ulcerogenic response to indomethacin has been demonstrated in rats (Takeuchi et al., 2002). Additionally, indomethacin decreased gastric mucosal PGE₂ content and produced gross pathological mucosal damage with gastric hypermotility and expression of COX-2 mRNA (Takeuchi et al., 2004). In the same study by Takeuchi et al. (2004), although SC-560 did not produce damage, it caused a decrease in the PGE₂ content and an increase in gastric motility as well as the up-regulation of COX-2 expression. Duodenal HCO₃⁻ secretion and luminal release of PGE₂ in rats were increased in response to mucosal acidification. This response was significantly inhibited by indomethacin but not by NS-398 or nimesulide (Hirata et al., 1997).

In addition to transporting ions, nutrients, and water, the intestinal epithelium, comprised of both enterocytes and their tight junctions (zona occludens), functions as a barrier that restricts the flow of luminal contents into the blood and lymphatics, and vice versa. In the small intestine, tight junctions are, on average, of the low-resistance type, meaning that most of the passive permeability of the epithelium to small monovalent ions and water resides in these junctional complexes (tight junctions in villi have higher resistance than do those in crypts). Colonic intercellular junctions are tighter, their resistance increasing steadily from proximal to distal portions (Field, 2003).

Although these junctions, which comprise a number of discrete proteins, are extracellular, their permeability properties are regulated by intracellular structures, especially actin filaments. Therefore, when the intestinal epithelium's barrier function is compromised by NSAID-associated erosions and ulcerations, loss of epithelial cells, and/or disruption of tight junctions, hydrostatic pressure in blood vessels and lymphatics will cause water and electrolytes, mucus, and protein to accumulate lumenally, leading to exudative diarrhea.

Effects on Physiological GI Mucosal Cell Renewal Mechanisms After Mucosal Injury

The integrity of the intestinal mucosal surface barrier is generally reestablished rapidly, even after extensive damage, due to its enormous regenerative capability.

The rapid-healing mechanism of the surface mucosal epithelium is accomplished by epithelial cell migration from proliferative zones into wound, also termed epithelial restitution, epithelial cell proliferation, and differentiation. This healing mechanism is regulated by a highly complex network of factors, such as regulatory peptides within the intestinal tract mucosa, conventionally designated as growth factors and cytokines. These factors play an essential role in regulating differential epithelial cell functions to preserve normal homeostasis and integrity of the intestinal mucosa (Dignass, 2001; Sturm and Dignass, 2008). Mucosal villous contraction and the restitution mechanism represent primary repair mechanisms in the GI tract which allow resealing of the epithelial barrier within minutes or hours via reformation of tight junctions between cells (Dignass, 2001; Sturm and Dignass, 2008). Villous contraction is initiated by myofibroblasts that reside immediately beneath the epithelial basement membrane. Subsequent events include crawling of healthy epithelium adjacent to the wound, referred to as restitution. Restitution is a well-coordinated event that is dependent on epithelial cell migration but independent of cell proliferation and differentiation. The structural integrity of the mucosa is maintained by continuous cell renewal from mucosal progenitor cells. This continuous renewal is a well-coordinated process that is controlled by proliferation of progenitor cells, which enables replacement of damaged or aged surface epithelial cells. Actin filaments, focal adhesions, and focal adhesion kinase (FAK) play crucial roles in the cell motility essential for restitution (Szabó et al., 2002).

Indomethacin significantly delayed epithelial restitution in rats and reduced FAK phosphorylation and recruitment to adhesion points, as well as actin stress fiber formation in migrating surface epithelial cells (Szabó et al., 2002). NSAIDs have been found to affect intestinal restitution through decreased potassium channel [K(v)1] surface expression and trafficking (Freeman et al., 2007). In an *in vitro* study, intestinal epithelial cell migration in response to wounding was reduced by indomethacin, phenylbutazone, and NS-398 but not by SC-560 (Freeman et al., 2007). NSAID inhibition of intestinal cell migration was not associated with depletion of intracellular polyamines (Freeman et al., 2007). However, another study, using pig small intestinal ileal mucosa, showed that endogenous PGs, released when mucosal injury occurs, mediate local repair of small intestinal epithelium after damage by the deconjugated bile salt deoxycholate. Whereas ongoing epithelial restitution and villous contraction were prominent features of repairing mucosa, acute recovery of barrier function was uniquely dependent on PG-mediated resealing of tight junctions and lateral intercellular space. The authors conclude that failure to repair increases in paracellular pathway permeability may underlie barrier failure resulting from NSAID use in patients with underlying enteropathy (Gookin et al., 2003).

A number of other factors, such as extracellular matrix, blood clotting factors, phospholipids, short-chain fatty acids, adenine nucleotides, trace elements, and calpain, have been demonstrated to modulate intestinal epithelial repair mechanisms (Dignass, 2001; Sturm and Dignass, 2008). Calpains, cysteine proteases, are involved in numerous cellular processes, such as cell migration and invasion. Altered expression of calpain proteins contributes to NSAID effects on intestinal

epithelial restitution. A multistep functional microarray genomic study was conducted using intestinal epithelial cells to identify novel signaling pathways that contribute to NSAID inhibition of GI epithelial cell migration (Raveendran et al., 2008). Raveendran et al. demonstrated that indomethacin and NS-398 decreased the expression of calpains 1, 2, and 8 proteins, whereas SC-560 had no effect on the expression of calpain proteins. Functional data were also consistent with decreased expression of calpain protein in cells treated with either NS-398 or indomethacin.

In gastric glands, a single stem cell in every gastric gland undergoes division to produce committed progenitor cells, which further differentiate into an adult epithelial cell type (Modlin et al., 2003). The stem/progenitor cell niche is made up of proliferating and differentiating epithelial cells and surrounding mesenchymal cells (Leedham et al., 2006). Intestinal subepithelial myofibroblasts (ISEMFs) are important coordinating cells that possess significant influence on their environment by virtue of their receptor profile and the signals they produce. Characteristically, ISEMFs form a protective fenestrated sheath around the stem cell compartment, creating the stem cell niche—the optimal microenvironment for stem cells to give rise to differentiated progeny (Leedham et al., 2006). The ISEMFs generate growth factors and thus promote mesenchymal-to-epithelial crosstalk and signaling to maintain the niche progenitor cell survival. Cell proliferation of progenitor cells is controlled by growth factors. The major growth factor receptor in rats during gastric ulcer healing, expressed in gastric progenitor cells, is the epidermal growth factor receptor (EGF-R) (Tarnawski et al., 1992), and the major mitogenic growth factors that activate this receptor are transforming growth factor alpha (TGF α) and insulin-like growth factor 1 (IGF-1) (Nguyen et al., 2007). EGF peptide itself is absent in normal gastric mucosa. However, it is present in the gastric lumen and can stimulate progenitor cell proliferation in case of injury. In human gastric epithelial monolayers, EGF treatment significantly stimulated cell migration and actin stress fiber formation, and increased FAK localization to focal adhesions, and phosphorylation of FAK and tensin (Szabó et al., 2002). In gastric ulcers in rats, IGF-1 promoted actin polymerization, cell proliferation, reepithelialization, and induced COX-2 in a phosphatidylinositol 3-kinase-dependent manner (Nguyen et al., 2007).

Both ns- and COX-2 s-NSAIDs may delay the healing of damaged GI mucosa. NSAIDs may delay healing after injury to the GI tract by affecting GI epithelium proliferation in response to mediators such as growth factors and nitric oxide (NO). The effects of celecoxib on normal and damaged (acid or ethanol challenged) gastric mucosa of rats were compared to those of ns-NSAIDs (Berenguer et al., 2004). In the absence of acid or ethanol challenge, only ns-NSAIDs produced appreciable gastric lesions. However, following acid or ethanol challenge, both COX-2 s- and ns-NSAIDs impaired healing of gastric mucosal damage. In rats, NS-398, a COX-2 s-NSAID, delayed gastric healing after acid-induced injury (Sun et al., 2000). Increased COX-2 expression was noted after growth stimulation by addition of serum which contains growth factors to cultured rat gastric mucosal cells *in vitro* and after acid-induced gastric injury to rats *in vivo* (Sawaoka et al., 1997; Horie-Sakata et al., 1998; Erickson et al., 1999; Sun et al., 2000). These

serum growth factors were TGF α or the hepatocyte growth factor (HGF) (Horie-Sakata et al., 1998; Sawaoka et al., 1999). Celecoxib did not alter the gastric mucosal barrier or induce mucosal lesions in healthy or NO-deficient rat gastric mucosa (Copelli et al., 2004). Celecoxib appeared to worsen the flare of the colon in a rat model of inflammatory bowel disease (Singh et al., 2004). In an isolated rabbit gastric epithelial cells model, aspirin significantly retarded wound healing, but simultaneous addition of growth factors such as IGF-I and EGF significantly accelerated wound repair (Yoshizawa et al., 2000). PGE₂ and gastrin transactivate EGF-R and trigger the mitogen-activated protein kinase pathway, thereby stimulating cell proliferation and exerting a trophic action on mucosa, resulting in gastric and intestinal hypertrophy (Pai et al., 2002). Therefore, NSAIDs are contraindicated in patients with damaged GI mucosa (peptic ulceration or GI bleeding).

Effects on Leukocyte Adhesion Molecules and Trafficking

Infiltration of leukocytes into the mucosa in response to initial tissue injury has been implicated in NSAID GI injury (Reuter et al., 1997). Leukocyte–endothelial cell interactions are mediated by various cell adhesion molecules. These interactions are important for leukocyte extravasation and trafficking in many pathological conditions in several body systems, including the GI tract. There are various stages of leukocyte trafficking into sites of inflammation. An initial slowing of leukocytes on the vascular endothelium is mediated by selectins. This event is followed by (1) activation of β_2 integrins after leukocyte exposure to cytokines and proinflammatory mediators, (2) adherence of leukocyte β_2 integrins to vascular endothelial ligands [e.g., intercellular adhesion molecule-1 (ICAM-1)], (3) extravasation of leukocytes into tissues through tight junctions of endothelial cells mediated by the platelet and endothelial cell adhesion molecule 1 (PECAM-1), and (4) perivascular migration through the extracellular matrix via β_1 integrins. Inhibiting excessive leukocyte egress and subsequent free-radical-mediated damage caused by leukocyte components may attenuate or eliminate tissue damage (Radi et al., 2001). Both piroxicam and meloxicam interfered, in an *in vitro* experiment using flow cytometry, with neutrophil degranulation and cytokine-mediated activation changes in adhesion molecules (García-Vicu na et al., 1997). Due to their anti-inflammatory properties, NSAIDs have been used to modify leukocyte infiltration via their effects on different stages of leukocyte trafficking in various animal models (Radi et al., 2001). For example, celecoxib and indomethacin inhibited leukocyte migration induced by lipopolysaccharide injected into the cremaster muscle in a rat model. However, celecoxib was associated with reduced leukocyte rolling and adhesion, whereas indomethacin only inhibited cell adhesion (Menezes et al., 2008). A role for leukocyte trafficking, adhesion, and activation in NSAID GI-mediated toxicity has been proposed. In a study in rats, neutrophil adhesion to gastric mesenteric venules was increased by indomethacin and celecoxib but not by SC-560, whereas gastric mucosal blood flow was decreased by indomethacin and SC-560 but not by celecoxib (Wallace et al., 2000). These data suggest that the NSAID-induced decrease in gastric mucosal blood flow is COX-1-mediated, whereas NSAID-induced neutrophil vascular endothelial adhesion is COX-2-mediated. In another study in rats,

neutrophil- and oxygen radical-dependent microvascular injuries were found to have a role in gastric mucosal injury induced by ns-NSAIDs. Also, reactive oxygen species (ROS) produced by activated neutrophils after indomethacin treatment in rats caused gastric mucosal injury via ROS-mediated oxidation of such macromolecules as lipids, proteins, and DNA (Naito and Yoshikawa, 2006).

Effects of GI Physiological Local pH, Gut Absorption, and Fasting

Absorption is a process whereby xenobiotics gain entrance to the body via the circulatory or lymphatic system. There are five possible processes of intestinal absorption of xenobiotics: (1) active transport, (2) passive diffusion, (3) pinocytosis, (4) filtration through “pores,” and (5) lymphatic absorption. Most xenobiotics are transported across the GI mucosa by passive diffusion (Chhabra, 1979). GI physiology can have a significant impact on absorption. A number of factors, such as diet, GI motility, interference with GI flora, changes in the rate of gastric emptying, age of the animal, physical properties of a compound, and the dissolution rate of xenobiotics can influence the rate of GI absorption (Chhabra, 1979). Lipid-soluble compounds are more readily absorbed than water-soluble compounds. Additionally, xenobiotic absorption is highly dependent on local GI pH values. However, there are considerable species differences in pH along each segment of the GI tract (Table 1-5) (Smith, 1965; Dressman et al., 1990; Davies and Morris, 1993; McConnell et al., 2008).

The pH in the GI tract is a crucial factor, affecting the stability and solubility of drugs and their absorption through the mucosa (McConnell et al., 2008). The gastric acid secretion rate in the dog at the basal state is low compared to that in humans and rhesus monkeys. In an *in vitro* experiment, Legen and Kristl (2003) demonstrated that ketoprofen transport across the rat jejunum has pH- and energy-dependent transport mechanisms. Indomethacin-induced gastric mucosal damage in rats is markedly dependent on luminal pH (Elliott et al., 1996). Acidic compounds can potentially cause gastric toxicity. Although ibuprofen is more potent than aspirin as a cyclooxygenase inhibitor, it has less gastric toxicity in animals and humans. This is because ibuprofen is 10 times less soluble under the acidic pH conditions

TABLE 1-5 Comparative pH Values of Various Anatomical Regions of the Gastrointestinal Tract

Species	Stomach	Small intestine	Cecum	Colon
Human	1.7–6.7	5.4–7.5	6	7.5
Monkey	2.8	5.6–6	5	5.1
Dog	3.4	6.2–7.5	6.4	6.5
Rat	3.2–3.9	5–6	5.9–6.6	5.5–6.2
Mouse	3–4	4.8–5.2	4.4–4.6	4.4–5
Rabbit	1.9	6–8	6.6	7.2

in the stomach and thus is unlikely to be absorbed there (Beck et al., 1990). Unsuitable pH may cause the precipitation of acidic or basic drugs from solution or the degradation of labile compounds. The lowest pH is seen in the stomach in laboratory animals and humans. However, the fasting status affects gastric pH values. In dogs and cats, gastric acid secretion is intermittent and gastric pH during fasting can rise as high as 3 to 6.5. In rodents, gastric pH appeared higher in the fasted state (McConnell et al., 2008). These observations are unlike that seen in humans, where the gastric pH is lower in the fasting state than in the fed state (fasted pH 1.7 increases to 6.7 after meal ingestion in humans) (Dressman et al., 1990). The fasting status affects NSAID GI-associated toxicity. For example, the gastric toxicity of indomethacin, diclofenac, ibuprofen, and aspirin at various doses is relatively low after oral administration to rats having access to food (Beck et al., 1990). On the other hand, fasted rats were more vulnerable to similar toxicity at all doses of these NSAIDs (Beck et al., 1990). The presence of food retards fenoprofen stomach absorption and lowers peak concentration in plasma, which is usually achieved within 2 h. In horses, food can impair the oral absorption of some NSAIDs, such as phenylbutazone. Phenylbutazone absorption from the GI tract in horses is influenced by the dose administered and the relationship of dosing to feeding. Access to hay can delay the time of peak plasma concentration to 18 h or longer (Tobin et al., 1986). The fed state of rodents, similar to the situation in humans, had no effect on intestinal pH (McConnell et al., 2008). The low intestinal pH level in rodents could have implications for the *in vivo* testing of oral drugs in rats and mice. For example, drugs that require a basic pH to dissolve may precipitate at the lower pH values seen in rodents. This suggests that rodent models may not be the most appropriate to use to study pH-sensitive dosage forms targeted to the human lower intestine and colon (McConnell et al., 2008).

A pH gradient has been reported at the gastric mucosal surface in several species (human, dog, rat, mouse, rabbit) (Johnson et al., 2006). This pH gradient is relatively alkaline directly at tissue surfaces and becomes more acidic at distances farther away from the surface. This alkaline layer is caused by active bicarbonate secretion and is considered a major defense mechanism. Duodenal hemorrhagic lesions were induced when pH was decreased (acid hypersecretion) (Hirata et al., 1997). In addition, such lesions were induced by histamine in rats (Hirata et al., 1997). In fact, histamine H₂-receptor antagonists have been used to reduce NSAID-induced gastric ulcers (Lazzaroni and Porro, 2009).

NSAID Topical Effect-Mediated Injury

Variation in the physicochemical properties and pharmacological profiles among the individual NSAIDs translates into interagent differences regarding the propensity to cause adverse GI effects (Bannwarth, 2008). It has been suggested that NSAID-associated GI hemorrhage and erosion are related to a topical effect of the drugs in addition to COX inhibition (Tibble et al., 2000). Enteric-coated or IV-injected drugs result in considerably less acute GI injury. In rats, the intestinal tolerability of celecoxib is thought to be related to the absence of a topical

damaging effect and COX-2 selective inhibition (Tibble et al., 2000). Topical GI mucosal injury usually occurs after ingestion of the weakly acidic and lipid-soluble NSAIDs. These weak acids are not ionized in the acidic gastric environment and their lipid solubility allows them to diffuse freely across the plasma membrane into surface epithelial cells. At cellular pH, they dissociate into the ionized form, releasing hydrogen ions that are trapped within the cell (intracellular trapping hypothesis), leading to an increase in back-diffusion of gastric acids and disruption of cellular function (Wolfe et al., 1999). Topical acidic properties can cause GI mucosal damage. For example, although sulindac is administered as a non-toxic prodrug, its active metabolite, sulindac sulfide, is excreted into bile. Upon entry into the duodenum, sulindac sulfide causes duodenal topical mucosal injury by virtue of its acidic properties (Wolfe et al., 1999). After aspirin ingestion, gastric mucosal permeability is increased, as reflected by a decrease in the transmucosal potential difference (Baskin et al., 1976). This is caused by a direct topical effect.

Changes in GI Motility, Microcirculation, and Enterobacteria

The inner layer of the GI tract, which is in intimate contact with the contents of the lumen, is comprised of an epithelial cell layer called the mucosa. Subjacent to the mucosa is the submucosa, which is a loose connective tissue layer that contains blood vessels, lymphatics, and autonomic nerve fibers (Meissner plexus). Beneath the submucosa are circular and longitudinal muscle layers comprised of smooth muscle fibers. Contraction of these muscles is associated with mixing and propulsive movements of the intestinal contents (GI peristalsis and motility). The intestinal motility is regulated by the enteric nervous system. GI inflammation (e.g., inflammatory bowel disease) generally affects its motility. The exact pathophysiology of this dysmotility is poorly understood. However, changes in myenteric neurons and smooth muscle have been proposed (Fornai et al., 2005, 2006). Emerging data suggest that cyclooxygenases may play a role in the control of GI neuromuscular functions and motility (De Backer et al., 2003; Fornai et al., 2005; 2006). Forani et al. reported that (1) in human colon, cyclooxygenases are involved in enteric circuitries exerting tonic inhibitory control on smooth muscle responses to endogenous acetylcholine; (2) both cyclooxygenase isoforms contribute to these regulatory actions; and (3) cholinergic neurons are modulated primarily by COX-1 activity, while COX-2 acts mainly at the muscular level to down-regulate muscarinic responses (Fornai et al., 2005). Gastric hypermotility occurred in indomethacin-treated rats (Takeuchi et al., 2004). Intestinal hypermotility has been implicated in NSAID-induced ulceration of the small intestine (Takeuchi et al., 2002). In an experimental study in rats, proximal duodenum motility was determined in the absence and presence of different NSAIDs. Treatment with rofecoxib at 5 mg/kg or parecoxib at 0.5 mg/kg induced duodenal motility, whereas SC-560 showed no effect (Pihl and Nylander, 2006). Another study demonstrated that COX-2 activation is a critical step in diminishing bowel propulsive motility in a trinitrobenzene sulfonic acid (TNBS)-induced colitis guinea pig model (Linden et al., 2004). In

this TNBS-induced colitis model, COX-2 inhibition with an s-NSAID, DFU, but not a COX-1 inhibitor, SC-560, restored to normal levels the electrical properties of myenteric neurons and the rate of propulsive motor activity (Linden et al., 2004). Thus, it is suggested that s-NSAIDs may be a possible therapeutic agent to improve bowel dysmotility.

GI mucosal and submucosal blood flow and microcirculation are important components of the gastroduodenal function and defense barrier. For example, in the stomach, the presence of luminal acid increases the delivery of vascular bicarbonate into the overlying mucus layer by mucosal microcirculation, thereby neutralizing H^+ ion invading from the lumen (Johnson et al., 2006). Indomethacin at an ulcerogenic dose of 25 mg/kg in rats enhances gastric motility and also induces microcirculatory disturbances at mucosal folds, which are caused by abnormal compression of the gastric mucosal wall and lead to increased microvascular permeability and cellular damage (Takeuchi et al., 1990). Wallace et al. (2000) reported that SC-560, but not celecoxib, produced a decrease in gastric mucosal blood flow in rats.

Enterobacterial invasion has also been implicated in NSAID-mediated GI pathophysiology. For example, the number of enterobacteria under both aerobic and anaerobic conditions is markedly increased in the intestinal mucosa following indomethacin treatment. Similarly, SC-560, with or without the coadministration of rofecoxib, increased the bacterial count in the mucosa, although rofecoxib alone did not (Takeuchi et al., 2010). The bacterial invasion in the intestinal mucosa following indomethacin treatment was blocked by prior administration of an ampicillin antibiotic, the numbers of bacteria being reduced even below control levels seen in the normal mucosa (Takeuchi et al., 2010). Enterobacteria and cytokines both play roles in the pathophysiology of NSAID-induced enteropathy. In addition, up-regulation of iNOS mRNA expression in the intestinal mucosa was observed in animals given SC-560 but not in animals given rofecoxib. Collectively, these data suggest that some NSAIDs cause GI hypermotility, followed by bacterial translocation, and GI microvascular disturbances, leading to the activation of neutrophils and expression of iNOS, and by doing so damage the intestine (Takeuchi et al., 2010).

Decreased Phosphatidylcholine Levels

A recent hypothesis related to the phosphatidylcholine (PC) role in the NSAID-associated toxicity mechanism has been proposed. Phosphatidylcholine is the major surfactant phospholipid that confers surface hydrophobic characteristics on the gastric mucosa. An improved safety profile of ibuprofen chemically associated with PC has been noted in elderly osteoarthritic patients (Lanza et al., 2008). It is possible that with age, surface phospholipid levels decrease below a critical threshold and that this reduction contributes to age-related NSAID intolerance (Lanza et al., 2008). In addition, age-associated decreases in surface hydrophobicity, PG levels, and impaired healing have been suggested to contribute to the deterioration of the barrier property of the gastric mucosa (Lanza et al., 2008). Hacklesberger et al.

(1998) observed a decrease in surface hydrophobicity in the antrum of the stomach, which is also one of the primary sites of NSAID-induced ulcers.

Impaired Drug Metabolism

Impaired drug metabolism with subsequent adverse drug effects can occur and is related to interindividual variability in drug metabolism due to polymorphisms in genes coded for drug-metabolizing enzymes such as cytochrome P450 (CYPs). Such impairment in drug metabolism would lead to increases in NSAID plasma concentrations and hence would increase the risk of developing adverse GI effects (Agúndez et al., 2009). Four NSAIDs—celecoxib, ibuprofen, lornoxicam, and piroxicam—are metabolized extensively by CYP2C9 and CYP2C8 enzymes, these enzymes being responsible for more than 90% of the primary metabolism of these drugs (Agúndez et al., 2009). Therefore, genetics has been suggested to predispose GI bleeding after NSAID use (Martinez et al., 2004). For example, inherited impairment in CYP2C9, an enzyme responsible for the metabolism of several NSAIDs, increases the risk for severe adverse drug reactions (i.e., GI bleeding) after NSAID use (Martinez et al., 2004; Pilotto et al., 2007). This suggests that CYP2C9 genotyping may identify subgroups of persons who are potentially at risk for NSAID-associated GI bleeding (Pilotto et al., 2007).

However, further investigation as to whether such GI bleeding is related to parent drugs or to metabolites is warranted. The impaired drug mechanism hypothesis would be relevant in long-term therapy because the drug would accumulate after multiple-dose exposure, but in many cases, patients with acute GI bleeding receive the NSAID only once, and the effect of an impaired metabolism in single-dose pharmacokinetics (PK) is less relevant, as can be expected in multiple-dose PK (Agúndez et al., 2009). An alternative mechanism for adverse GI drug effects that should be explored is whether impaired function of the main enzymes could drive the metabolism of the NSAIDs to alternative metabolic pathways and whether alternative metabolites may participate in the adverse effects (Agúndez et al., 2009).

Role of Toll-like Receptor (TLR)-4/MyD88 and Enteric Bacteria

Toll-like receptors (TLRs) comprise a family of conserved molecular structures (pathogen-associated molecular patterns) that function as sensors of microbial infection and play a central role in mucosal innate immune regulation (Rakoff-Nahoum et al., 2004). TLR activation leads to the production of cytokines and antimicrobial molecules important in the initial innate immune response. However, in addition to their function in host defense, recent findings indicate that activation of TLRs by commensal microflora is critical for protection against GI injury and associated mortality. Therefore, TLRs appear to control intestinal epithelial homeostasis and protection from injury (Rakoff-Nahoum et al., 2004). TLR-4 recognizes lipopolysaccharide (LPS), which is present in the cell wall of gram-negative bacteria. The interaction of LPS with TLR-4 and its coreceptor, MD-2, triggers signaling cascades mediated via the accessory protein MyD88-dependent pathways that lead

to translocation of the transcription factor nuclear factor κ B (NF κ B) and the production of proinflammatory cytokines (Medzhitov et al., 1998). TLR-4 signaling is important in the recruitment of inflammatory cells and the production of inflammatory cytokines in the intestine. In a mouse model of inflammatory bowel disease, TLR-4 was found to mediate PGE₂ production by regulation of COX-2 (Fukata et al., 2005, 2006). TLR-4 deficiency (TLR-4^{-/-}) in mice results in fewer inflammatory infiltrates in the lamina propria (Fukata et al., 2005). Furthermore, a study in mice assessed the role of TLR-4 activation and signaling through MyD88 in intestinal ischemia/reperfusion (I/R)-induced damage at 2 h postischemia (Moses et al., 2009). The investigators found that a lack of TLR-4 or MyD88 attenuated intestinal damage to approximately 50% of that seen in wild-type mice. The attenuated gut injury was accompanied by decreased proinflammatory mediators, including chemokines, cytokines, and PGE₂ production. The decreased PGE₂ appeared to be mediated by COX-2 activation (Moses et al., 2009). These studies support the hypothesis that TLR-4 expression affects the extent of intestinal damage by altering COX-2-mediated PGE₂ production. However, PGE₂ alone was not sufficient to restore damage in the TLR-4-altered mice, implicating additional mechanisms of TLR-4-mediated damage. Thus, these data indicate that TLR-4 stimulation of COX-2 activation of PGE₂ production is necessary but not sufficient for intestinal I/R-induced damage and inflammation. TLR-4^{-/-} mice also had defects in mucosal repair in response to dextran sodium sulfate (DSS)-induced colitis with decreased epithelial proliferation and increased rectal bleeding (Fukata et al., 2006). Collectively, these studies suggest that TLR-4 signaling may serve a dual role in the GI tract as a mediator of both inflammation and mucosal repair (Ungaro et al., 2009).

Intestinal epithelial cells showed up-regulated COX-2 expression in a TLR-4- and MyD88-dependent fashion in the DSS colitis mouse model (Fukata et al., 2006). TLR-4 has been implicated in ns-NSAID-induced (indomethacin) small intestine damage because TLR-4^{-/-} and MyD88^{-/-} mice showed resistance to ns-NSAID-induced small intestinal damage (Watanabe et al., 2008). The physiological importance of PGs in intestinal epithelial cells and its relationship to bacterial invasion have been demonstrated. In vitro infection of human intestinal epithelial cells with invasive bacteria has been shown to induce the expression of PGHS₂ and the production of PGE₂ and PGF_{2 α} (Eckmann et al., 1997). Furthermore, increased PGHS-2 expression was observed in intestinal epithelial cells in vivo after infection with invasive bacteria using a human intestinal xenograft model in SCID mice (Eckmann et al., 1997). The bacterial LPS/TLR-4 signaling pathway is also a key mechanism in NSAID-induced enteropathy. Elevation in enteric bacterial numbers and epithelial permeability of the small intestine was associated with enterohepatically recirculated ns-NSAIDs (Reuter et al., 1997). Another study investigated the effects of the *Lactobacillus casei* strain Shirota (LcS) on indomethacin-induced small intestine injury in rats. One-week treatment with viable LcS prevented indomethacin-induced intestinal injury. The investigators conclude that LcS exhibited a prophylactic effect on indomethacin-induced enteropathy by suppressing the LPS/TLR-4 signaling pathway (Watanabe et al.,

2009). Bacterial invasion in the intestinal mucosa following indomethacin treatment in rats was blocked by prior administration of the ampicillin antibiotic, the numbers of bacteria being reduced even below control levels seen in the normal mucosa (Takeuchi et al., 2010).

Role of Uncoupling of Mitochondrial Oxidative Phosphorylation

Several studies examined the role of the uncoupling of mitochondrial oxidative phosphorylation, leading to increased intestinal permeability and calcium release into the cytosol, in NSAID-mediated GI effects (Mahmud et al., 1996; Mathews, 1996; Somasundaram et al., 2000). NSAIDs increase mitochondrial respiration in vivo and in vitro without producing ATP. The higher pH inside the mitochondrial matrix deprotonates the NSAID, so protons are transported into the matrix. NSAIDs that directly uncouple or inhibit mitochondrial oxidative phosphorylation and ATP turnover include indomethacin, aspirin, diclofenac, meloxicam, and SC-236, an s-NSAID (Petrescu et al., 1997; Tibble et al., 2000; Krause et al., 2003). For example, indomethacin, but not celecoxib, uncoupled mitochondrial oxidative phosphorylation both in vitro and in vivo, caused a significant increase in small intestinal permeability, caused mucosal inflammation and a 90% decline in intestinal PGE levels, and was associated with multiple small intestinal ulcers in rats (Tibble et al., 2000). Sulindac sulfide, but not sulindac sulfone or sulindac itself, caused mitochondrial uncoupling in an isolated rat liver mitochondria (Leite et al., 2006). However, while the uncoupling of enterocyte mitochondrial oxidative phosphorylation leads to increased intestinal permeability and low-grade inflammation, concurrent decreases in mucosal prostanoids appear to be important in the development of ulcers (Somasundaram et al., 2000).

Role of Peroxisome Proliferator-Activated Receptor γ

Peroxisome proliferator-activated receptor γ (PPAR γ) is a ligand-activated nuclear receptor whose activation has been linked to several pathways, including regulation of intestinal inflammation (Issemann and Green, 1990). COX-2 is elaborated during I/R injury (Sato et al., 2005). Issemann and Green examined the importance of PPAR γ in inflammation and GI I/R-induced injury using a PPAR γ -knockout mouse model. PPAR γ -knockout mice showed exacerbated GI I/R-induced injury compared to wild-type mice. Histopathological examination of the small intestine revealed loss of villi (erosions and ulcers), hemorrhage, and inflammatory cell infiltrates. Furthermore, PPAR γ activation reduced the severity of GI I/R injury and blocked up-regulation of NF κ B, which is involved in the control of transcription of various inflammatory genes, such as TNF α (Issemann and Green, 1990). In a rodent model of I/R, NS-398, a COX-2 s-NSAID, reversed small intestine inflammation and injury and induced expression and nuclear translocation of PPAR γ (Sato et al., 2005). In addition, several ns-NSAIDs (e.g., naproxen, ibuprofen, indomethacin, fenpropfen) activate PPAR γ (Jaradat et al., 2001). In a porcine I/R model, injured ileum treated with NS-398, an s-NSAID, recovered to control levels within 3 h

(Blikslager et al., 2002). Therefore, PPAR γ appears to play a role in NSAID-mediated GI pathophysiological cellular mechanisms, and NSAID administration appears to have a protective effect in I/R GI conditions.

Role of Mitogen-Activated Protein Kinases

The mitogen-activated protein kinases (MAPKs) transduce a variety of extracellular signals to the transcription machinery and include three distinct mammalian types—extracellular signal-regulated kinases (ERKs), c-Jun NH₂-terminal kinases (JNKs), and p38 MAPKs (p38)—the latter having four isoforms of its own (α , β , γ , δ) (Radi and Khan, 2006a; Radi et al., 2009). p38 MAPKs have been shown to be crucial for COX-2 expression and PPAR γ (Scherle et al., 2000; Radi et al., 2009). In fact, the p38 MAPK pathway can activate the intestinal epithelial cells *cox-2* gene promoter directly (Grishin et al., 2006). It has been found that MAPKs regulate COX-2 expression and mucosal recovery in an in vitro porcine I/R ileum model (Shifflett et al., 2004). This suggests that the MAPK pathway can have a positive regulatory effect on COX expression in I/R GI conditions, which suggests that NSAID administration would be protective under such conditions.

NSAID GI Injury-Associated Risk Factors

Epidemiological studies suggest that there are several risk factors for the development of NSAID-associated GI events in humans. These include advancing age, a high dose of NSAID, use of more than two NSAIDs, concurrent paracetamol, concurrent anticoagulants, concurrent aspirin, and prior history of peptic ulcer disease, high alcohol consumption, cigarette smoking, and *H. pylori* infection (Rainsford, 2009).

CONCLUSIONS

The comparative pathophysiological aspects of the GI tract and interspecies COX-1 and COX-2 expression levels and the pathophysiological role of cyclooxygenases (COX-1 and COX-2) and the effects of their inhibition in the GI system are discussed in this chapter. There are significant interspecies differences in both the level of COX-1 expression and the ratio of COX-1 and COX-2 expression and susceptibility to toxicity with COX inhibition in the GI tract. Nonselective NSAIDs are used to treat a variety of inflammatory disease conditions. The ns-NSAIDs have been associated with GI toxicity in many species. Examples of GI-related toxicities are bleeding, ulceration, erosions, and perforations, distributed across the pyloric region, gastric mucosa, jejunum, ileum, duodenum, and cecum. COX-2 s-NSAIDs have a superior and improved GI tolerability profile to that of ns-NSAIDs. The analgesic and anti-inflammatory benefits of NSAIDs are linked to COX-2 inhibition, while many of the GI toxicities and side effects have variably been linked to COX-1 and/or COX-2 inhibition and, in some cases, directly to the secondary pharmacologic properties of the select drugs. Several mechanisms involved in

the pathogenesis of these NSAID-associated toxicities include differences in COX enzyme potency, interspecies anatomical differences, changes in GI motility, aging, fasting status, disruption of GI physiologic mucosal defense mechanisms, effects on the physiologic GI mucosal cell renewal mechanisms, alterations in GI physiologic pH secretion regulation, inhibition of PG synthesis, impaired drug metabolism, effects of the enterohepatic recirculation, decreased phosphatidylcholine levels, the role of TLR-4/MyD88, PPARs, MAPKs, and glucuronidation, neutrophil adherence, and direct chemical damage in the GI tract. COX-2 s-NSAIDs have permitted comparable therapeutic benefit to conventional ns-NSAIDs without these attendant COX-1-mediated toxicities (Radi and Khan, 2006b).

REFERENCES

- Adams SS, Bough RG, Cliffe EE, Lessel B, et al. Absorption, distribution and toxicity of ibuprofen. *Toxicol Appl Pharmacol* 1969;15:310–330.
- Agrawal NM, Aziz K. Prevention of gastrointestinal complications associated with nonsteroidal antiinflammatory drugs. *J Rheumatol Suppl* 1998;51:17–20.
- Agúndez JA, García-Martín E, Martínez C. Genetically based impairment in CYP2C8- and CYP2C9-dependent NSAID metabolism as a risk factor for gastrointestinal bleeding: Is a combination of pharmacogenomics and metabolomics required to improve personalized medicine? *Expert Opin Drug Metab Toxicol* 2009;5:607–620.
- Aguwa CN. Incidence of gastric ulcers by indomethacin and piroxicam in rats. *Arch Toxicol* 1985;56:212–213.
- Aitken MM, Sanford J. Plasma levels following administration of sodium meclizemate by various routes. *Res Vet Sci* 1975;19:241–244.
- al-Ghamdi MS, Dissanayake AS, Cader ZA, Jain S. Tenoxicam-induced gastropathy in the rat: a comparison with piroxicam and diclofenac sodium, and the inhibitory effects of ranitidine and sucralfate. *J Int Med Res* 1991;19:242–248.
- Allison SO, Halliday LC, French JA, Novikov DD, et al. Assessment of buprenorphine, carprofen, and their combination for postoperative analgesia in olive baboons (*Papio anubis*). *J Am Assoc Lab Anim Sci* 2007;46:24–231.
- Altinkaynak K, Suleyman H, Akcay F. Effect of nimesulide, rofecoxib and celecoxib on gastric tissue glutathione level in rats with indomethacin-induced gastric ulcerations. *Pol J Pharmacol* 2003;55:645–648.
- Anthony A, Dhillon AP, Sim R, Nygard G, et al. Ulceration, fibrosis and diaphragm-like lesions in the caecum of rats treated with indomethacin. *Aliment Pharmacol Ther* 1994;8:417–424.
- Aoyagi N, Ogata H, Kaniwa N, Uchiyama M, et al. Gastric emptying of tablets and granules in humans, dogs, pigs, and stomach-emptying-controlled rabbits. *J Pharm Sci* 1992;81:1170–1174.
- Arai I, Hamasaka Y, Futaki N, Takahashi S, et al. Effect of NS-398, a new nonsteroidal anti-inflammatory agent, on gastric ulceration and acid secretion in rats. *Res Commun Chem Pathol Pharmacol* 1993;81:259–270.
- Atchison CR, West AB, Balakumaran A, Hargus SJ, et al. Drug enterocyte adducts: possible causal factor for diclofenac enteropathy in rats. *Gastroenterology* 2000;119:1537–1547.
- Atzpodien E, Mehdi N, Clarke D, Radhofer-Welte S. Subacute and chronic oral toxicity of lornoxicam in cynomolgus monkeys. *Food Chem Toxicol* 1997;35:465–474.
- Bannwarth B. Safety of the nonselective NSAID nabumetone: focus on gastrointestinal tolerability. *Drug Saf* 2008;31:485–503.
- Baskin WN, Ivey KJ, Krause WJ, Jeffrey GE, et al. Aspirin-induced ultrastructural changes in human gastric mucosa: correlation with potential difference. *Ann Intern Med* 1976;85:299–303.
- Beck WS, Schneider HT, Dietzel K, Nuernberg B, et al. Gastrointestinal ulcerations induced by anti-inflammatory drugs in rats: physicochemical and biochemical factors involved. *Arch Toxicol* 1990;64:210–217.

- Berenguer B, Alarcón de la Lastra C, Motilva V, La Casa C, et al. Effects of celecoxib on acid-challenged gastric mucosa of rats: comparison with metamizol and piroxicam. *Dig Dis Sci* 2004;49:937–947.
- Bhandari P, Bateman AC, Mehta RL, Patel P. Mucosal expression of cyclooxygenase isoforms 1 and 2 is increased with worsening damage to the gastric mucosa. *Histopathology* 2005;46:280–286.
- Bjorkman DJ. Nonsteroidal anti-inflammatory drug-induced gastrointestinal injury. *Am J Med* 1996;101:25S–32S.
- Blikslager AT, Zimmer DN, Young KM, Campbell NB, et al. Recovery of ischaemic injured porcine ileum: evidence for a contributory role of COX-1 and COX-2. *Gut* 2002;50:615–623.
- Bombardier C, Laine L, Reicin A, Shapiro D, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med* 2000;343:1520–1528.
- Bowen B, Yuan Y, James C, Rashid F, et al. Time course and pattern of blood loss with ibuprofen treatment in healthy subjects. *Clin Gastroenterol Hepatol* 2005;3:1075–1082.
- Brodie DA, Cook PG, Bauer BJ, Dagle GE. Indomethacin-induced intestinal lesions in the rat. *Toxicol Appl Pharmacol* 1970;17:615–624.
- Budsberg SC, Johnston SA, Schwarz PD, DeCamp CE, et al. Efficacy of etodolac for the treatment of osteoarthritis of the hip joints in dogs. *J Am Vet Med Assoc* 1999;214:206–210.
- Burdan F, Szumiło J, Gajjar B, Dudka J, et al. Immunoexpression of constitutive and inducible cyclo-oxygenase isoforms in the rat foetal and maternal digestive tract. *Folia Morphol (Warsz)* 2008;67:24–31.
- Campbell NB, Blikslager AT. The role of cyclooxygenase inhibitors in repair of ischaemic-injured jejunal mucosa in the horse. *Equine Vet J* 2000;32:59–64.
- Carrick JB, Papich MG, Middleton DM, Naylor JM, et al. Clinical and pathological effects of flunixin meglumine administration to neonatal foals. *Can J Vet Res* 1989;53:195–201.
- Cathers TE, Isaza R, Oehme F. Acute ibuprofen toxicosis in a ferret. *J Am Vet Med Assoc* 2000;216:1426–1428.
- Chan CC, Boyce S, Brideau C, Charleson S, et al. Rofecoxib [Vioxx, MK-0966; 4-(4'-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor—pharmacological and biochemical profiles. *J Pharmacol Exp Ther* 1999;290:551–560.
- Chhabra RS. Intestinal absorption and metabolism of xenobiotics. *Environ Health Perspect* 1979;33:61–69.
- Collins LG, Tyler DE. Experimentally induced phenylbutazone toxicosis in ponies: description of the syndrome and its prevention with synthetic prostaglandin E2. *Am J Vet Res* 1985;46:1605–1615.
- Combe B, Swergold G, McLay J, McCarthy T, et al. Cardiovascular safety and gastrointestinal tolerability of etoricoxib vs. diclofenac in a randomized controlled clinical trial (the MEDAL study). *Rheumatology (Oxford)* 2009;48:425–432.
- Conforti A, Donini M, Brocco G, Del Soldato P, et al. Acute anti-inflammatory activity and gastrointestinal tolerability of diclofenac and nitrofenac. *Agents Actions* 1993;40:176–180.
- Conlon PD. Nonsteroidal drugs used in the treatment of inflammation. *Vet Clin North Am Small Anim Pract* 1988;18:1115–1131.
- Cook VL, Meyer CT, Campbell NB, Blikslager AT. Effect of firocoxib or flunixin meglumine on recovery of ischemic-injured equine jejunum. *Am J Vet Res* 2009;70:992–1000.
- Coppelli G, Guaita E, Spaggiari S, Coruzzi G, et al. Gastric effects of the selective cyclooxygenase-2 inhibitor, celecoxib, in the rat. *Dig Liver Dis* 2004;36:265–370.
- Cosenza SF. Drug-induced gastroduodenal ulceration in dogs. *Modern Vet Pract* 1984;65:923–925.
- Court MH, Greenblatt DJ. Molecular basis for deficient acetaminophen glucuronidation in cats: an interspecies comparison of enzyme kinetics in liver microsomes. *Biochem Pharmacol* 1997;53:1041–1047.
- Cryer B. Gastrointestinal safety of low-dose aspirin. *Am J Manag Care* 2002;8:S701–S708.
- Cryer B, Feldman M. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. *Am J Med* 1998;104:413–421.
- Cryer B, Redfern JS, Goldschmidt M, Lee E, et al. Effect of aging on gastric and duodenal mucosal prostaglandin concentrations in humans. *Gastroenterology* 1992;102:1118–1123.

- Cuzzocrea S, Mazzon E, Serraino I, Dugo L, et al. Celecoxib, a selective cyclooxygenase-2 inhibitor reduces the severity of experimental colitis induced by dinitrobenzene sulfonic acid in rats. *Eur J Pharmacol* 2001;431:91–102.
- Daehler MH. Transmural pyloric perforation associated with naproxen administration in a dog. *J Am Vet Med Assoc* 1986;189:694–695.
- Daffonchio L, Novellini R, Bertuglia S. Protective effect of ketoprofen lysine salt on interleukin-1beta and bradykinin induced inflammatory changes in hamster cheek pouch microcirculation. *Inflamm Res* 2002;51:223–228.
- Dannenberg AJ, Altorki NK, Boyle JO, Dang C, et al. Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. *Lancet Oncol* 2001;2:544–551.
- Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993;10:1093–1095.
- De Backer O, Leclere PG, Lefebvre RA. Pharmacological characterization of pre- and postsynaptic prostanoid receptors in pig gastric fundus. *Neuropharmacology* 2003;45:684–690.
- Deng X, Tolstanova G, Khomenko T, Chen L, et al. Mesalamine restores angiogenic balance in experimental ulcerative colitis by reducing expression of endostatin and angiostatin: novel molecular mechanism for therapeutic action of mesalamine. *J Pharmacol Exp Ther* 2009;331:1071–1078.
- Dequeker J, Hawkey C, Kahan A, Steinbrück K, et al. Improvement in gastrointestinal tolerability of the selective cyclooxygenase (COX)-2 inhibitor, meloxicam, compared with piroxicam: results of the safety and efficacy large-scale evaluation of COX-inhibiting therapies (SELECT) trial in osteoarthritis. *Br J Rheumatol* 1998;37:946–951.
- Dignass AU. Mechanisms and modulation of intestinal epithelial repair. *Inflamm Bowel Dis* 2001;7:68–77.
- DiPasquale G, Welaj P. Letter: Ulcerogenic potential of indomethacin in arthritic and non-arthritic rats. *J Pharm Pharmacol* 1973;25:831–832.
- Doan T, Massarotti E. Rheumatoid arthritis: an overview of new and emerging therapies. *J Clin Pharmacol* 2005;45:751–762.
- Doucet MY, Bertone AL, Hendrickson D, Hughes F, et al. Comparison of efficacy and safety of paste formulations of firocoxib and phenylbutazone in horses with naturally occurring osteoarthritis. *J Am Vet Med Assoc* 2008;232:91–97.
- Dressman JB, Berardi RR, Dermentzoglou LC, Russell TL, et al. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm Res* 1990;7:756–761.
- Duerksen DR. Stress-related mucosal disease in critically ill patients. *Best Pract Res Clin Gastroenterol* 2003;17:327–244.
- Duggan DE, Hooke KF, Noll RM, Kwan C. Enterohepatic circulation of indomethacin and its role in intestinal irritation. *Biochem Pharmacol* 1975;24:1749–1754.
- Eckmann L, Stenson WF, Savidge TC, Lowe DC, et al. Role of intestinal epithelial cells in the host secretory response to infection by invasive bacteria: Bacterial entry induces epithelial prostaglandin H synthase-2 expression and prostaglandin E2 and F2alpha production. *J Clin Invest* 1997;100:296–309.
- Eling TE, Thompson DC, Foureman GL, Curtis JF, et al. Prostaglandin H synthase and xenobiotic oxidation. *Annu Rev Pharmacol Toxicol* 1990;30:1–45.
- Elliott GA, Purmalis A, Vandermeer DA, Denlinger RH. The propionic acids: gastrointestinal toxicity in various species. *Toxicol Pathol* 1988;16:245–250.
- Elliott SL, Ferris RJ, Giraud AS, Cook GA, et al. Indomethacin damage to rat gastric mucosa is markedly dependent on luminal pH. *Clin Exp Pharmacol Physiol* 1996;23:432–434.
- El-Medany A, Mahgoub A, Mustafa A, Arafa M, et al. The effects of selective cyclooxygenase-2 inhibitors, celecoxib and rofecoxib, on experimental colitis induced by acetic acid in rats. *Eur J Pharmacol* 2005;507:291–299.
- Erickson BA, Longo WE, Panesar N, Mazuski JE, et al. The effect of selective cyclooxygenase inhibitors on intestinal epithelial cell mitogenesis. *J Surg Res* 1999;81:101–107.
- Ewing GO. Indomethacin-associated gastrointestinal hemorrhage in a dog. *J Am Vet Med Assoc* 1972;161:1665–1668.
- Eyre P, Boulard C, Deline T. Local and systemic reactions in cattle to *Hypoderma lineatum* larval toxin: protection by phenylbutazone. *Am J Vet Res* 1981;42:25–28.

- Fang WF, Broughton A, Jacobson ED. Indomethacin-induced intestinal inflammation. *Am J Dig Dis* 1977;22:749–760.
- Feldman M, Cryer B. Effects of age on gastric alkaline and nonparietal fluid secretion in humans. *Gerontology* 1998;44:222–227.
- Field M. Intestinal ion transport and the pathophysiology of diarrhea. *J Clin Invest* 2003;111:931–943.
- Fornai M, Blandizzi C, Colucci R, Antonioli L, et al. Role of cyclooxygenases 1 and 2 in the modulation of neuromuscular functions in the distal colon of humans and mice. *Gut* 2005;54:608–616.
- Fornai M, Blandizzi C, Antonioli L, Colucci R, et al. Differential role of cyclooxygenase 1 and 2 isoforms in the modulation of colonic neuromuscular function in experimental inflammation. *J Pharmacol Exp Ther* 2006;317:938–945.
- Forsyth SF, Guilford WG, Haslett SJ, Godfrey J. Endoscopy of the gastroduodenal mucosa after carprofen, meloxicam and ketoprofen administration in dogs. *J Small Anim Pract* 1998;39:421–424.
- Fox J, Anderson LC, Loew FM, Quimby FW. *Laboratory Animal Medicine*, 2nd ed. San Diego, CA: Academic Press, 2002.
- Fox SM, Johnston SA. Use of carprofen for the treatment of pain and inflammation in dogs. *J Am Vet Med Assoc* 1997;210:1493–1498.
- Freeman LC, Narvaez DF, McCoy A, von Stein FB, et al. Depolarization and decreased surface expression of K channels contribute to NSAID-inhibition of intestinal restitution. *Biochem Pharmacol* 2007;74:74–85.
- Fukata M, Michelsen KS, Eri R, Thomas LS, et al. Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *Am J Physiol Gastrointest Liver Physiol* 2005;288:G1055–G1065.
- Fukata M, Chen A, Klepper A, Krishnareddy S, et al. Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: role in proliferation and apoptosis in the intestine. *Gastroenterology* 2006;131:862–877.
- Futaki N, Yoshikawa K, Hamasaka Y, Arai I, et al. NS-398, a novel non-steroidal anti-inflammatory drug with potent analgesic and antipyretic effects, which causes minimal stomach lesions. *Gen Pharmacol* 1993;24:105–110.
- García-Vicuña R, Díaz-González F, González-Alvaro I, del Pozo MA, et al. Prevention of cytokine-induced changes in leukocyte adhesion receptors by nonsteroidal antiinflammatory drugs from the oxicam family. *Arthritis Rheum* 1997;40:143–153.
- Girawan D, Abdurachman SA, Djumhana A, Roslia J, et al. Comparison of endoscopic gastric mucosa features after administration of piroxicam to meloxicam and their correlation with dyspepsia symptoms in elderly patient with knee osteoarthritis. *Acta Med Indones* 2004;36:202–206.
- Godshalk CP, Roush JK, Fingland RB, Sikkema D, et al. Gastric perforation associated with administration of ibuprofen in a dog. *J Am Vet Med Assoc* 1992;201:1734–1736.
- Goldstein JL, Correa P, Zhao WW, Burr AM, et al. Reduced incidence of gastroduodenal ulcers with celecoxib, a novel cyclooxygenase-2 inhibitor, compared to naproxen in patients with arthritis. *Am J Gastroenterol* 2001;96:1019–1027.
- Goldstein JL, Aisenberg J, Lanza F, Schwartz H, et al. A multicenter, randomized, double-blind, active-comparator, placebo-controlled, parallel-group comparison of the incidence of endoscopic gastric and duodenal ulcer rates with valdecoxib or naproxen in healthy subjects aged 65 to 75 years. *Clin Ther* 2006;28:340–351.
- Goldstein JL, Eisen GM, Lewis B, Gralnek IM, et al. Small bowel mucosal injury is reduced in healthy subjects treated with celecoxib compared with ibuprofen plus omeprazole, as assessed by video capsule endoscopy. *Aliment Pharmacol Ther* 2007;25:1211–1222.
- Goodman L, Torres B, Punke J, Reynolds L, et al. Effects of firocoxib and tepoxalin on healing in a canine gastric mucosal injury model: effects of firocoxib and tepoxalin on healing in a canine gastric mucosal injury model. *J Vet Intern Med* 2009;23:56–62.
- Goodman LA, Torres BT, Reynolds LR, Budsberg SC. Effects of firocoxib, meloxicam, and tepoxalin administration on eicosanoid production in target tissues of healthy cats. *Am J Vet Res* 2010;71:1067–1073.
- Gookin JL, Galanko JA, Blikslager AT, Argenzio RA. PG-mediated closure of paracellular pathway and not restitution is the primary determinant of barrier recovery in acutely injured porcine ileum. *Am J Physiol Gastrointest Liver Physiol* 2003;285:G967–G979.

- Goto H, Sugiyama S, Ohara A, Hoshino H, et al. Age-associated decreases in prostaglandin contents in human gastric mucosa. *Biochem Biophys Res Commun* 1992;186:1443–1448.
- Gretzer B, Maricic N, Respondek M, Schuligoi R, et al. Effects of specific inhibition of cyclooxygenase-1 and cyclo-oxygenase-2 in the rat stomach with normal mucosa and after acid challenge. *Br J Pharmacol* 2001;132:1565–1573.
- Grishin AV, Wang J, Potoka DA, Hackam DJ, et al. Lipopolysaccharide induces cyclooxygenase-2 in intestinal epithelium via a noncanonical p38 MAPK pathway. *J Immunol* 2006;176:580–588.
- Gullikson GW, Anglin CP, Kessler LK, Smeach S, et al. Misoprostol attenuates aspirin-induced changes in potential difference and associated damage in canine gastric mucosa. *Clin Invest Med* 1987;10:145–151.
- Hackelsberger A, Platzer U, Nilius M, Schultze V, et al. Age and *Helicobacter pylori* decrease gastric mucosal surface hydrophobicity independently. *Gut* 1998;43:465–469.
- Hallesy DW, Shott LD, Hill R. Comparative toxicology of naproxen. *Scand J Rheumatol* 1973;Suppl 2: 220–228.
- Harris SI, Stoltz RR, LeComte D, Hubbard RC. Parecoxib sodium demonstrates gastrointestinal safety comparable to placebo in healthy subjects. *J Clin Gastroenterol* 2004;38:575–580.
- Hawkey C, Kahan A, Steinbrück K, Alegre C, et al. Gastrointestinal tolerability of meloxicam compared to diclofenac in osteoarthritis patients. International MELISSA Study Group: Meloxicam Large-Scale International Study Safety Assessment. *Br J Rheumatol* 1998;37:937–945.
- Hawkey C, Laine L, Simon T, Beaulieu A, et al. Comparison of the effect of rofecoxib (a cyclooxygenase 2 inhibitor), ibuprofen, and placebo on the gastroduodenal mucosa of patients with osteoarthritis: a randomized, double-blind, placebo controlled trial. The Rofecoxib Osteoarthritis Endoscopy Multi-national Study Group. *Arthritis Rheum* 2000;43:370–377.
- Hawkey CJ, Skelly MM. Gastrointestinal safety of selective COX-2 inhibitors. *Curr Pharm Des* 2002;8:1077–1089.
- Haworth R, Oakley K, McCormack N, Pilling A. Differential expression of COX-1 and COX-2 in the gastrointestinal tract of the rat. *Toxicol Pathol.* 2005;33:239–245.
- Hirata T, Ukawa H, Kitamura M, Takeuchi K. Effects of selective cyclooxygenase-2 inhibitors on alkaline secretory and mucosal ulcerogenic responses in rat duodenum. *Life Sci* 1997;61:1603–1611.
- Horie-Sakata K, Shimada T, Hiraishi H, Terano A. Role of cyclooxygenase 2 in hepatocyte growth factor-mediated gastric epithelial restitution. *J Clin Gastroenterol* 1998;27 Suppl 1:S40–S46.
- Hull MA, Booth JK, Tisbury A, Scott N, et al. Cyclooxygenase 2 is up-regulated and localized to macrophages in the intestine of Min mice. *Br J Cancer* 1999;79:1399–1405.
- Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990;347:645–650.
- Jackson LM, Wu KC, Mahida YR, Jenkins D, et al. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. *Gut* 2000;47:762–770.
- Jaradat MS, Wongsud B, Phornchirasilp S, Rangwala SM, et al. Activation of peroxisome proliferator-activated receptor isoforms and inhibition of prostaglandin H(2) synthases by ibuprofen, naproxen, and indomethacin. *Biochem Pharmacol* 2001;62:1587–1595.
- Johnson LR, Barret KE, Gishan FK, Merchant JL, Said HM, Wood JD (eds). *Physiology of the Gastrointestinal Tract*, 4th ed. New York: Elsevier, 2006, pp. 1259–1291.
- Johnston SA, Budsberg SC. Nonsteroidal anti-inflammatory drugs and corticosteroids for the management of canine osteoarthritis. *Vet Clin North Am Small Anim Pract* 1997;27:841–862.
- Johnston SA, Leib MS, Forrester SD, Marini M. The effect of misoprostol on aspirin-induced gastroduodenal lesions in dogs. *J Vet Intern Med* 1995;9:32–38.
- Julou L, Guyonnet JC, Ducrot R, Fournel J, et al. Ketoprofen (19.583R.P.) [2-(3-benzoylphenyl)propionic acid]. Main pharmacological properties: line of toxicological and pharmacokinetic data. *Scand J Rheumatol* 1976;1976:33–44.
- Kallings P, Johnston C, Drevemo S. Effects of flunixin on movement and performance of standardbred trotters on the track. *Equine Vet J* 1999;Suppl 30: 270–273.
- Karcher LF, Dill SG, Anderson WI, King JM. Right dorsal colitis. *J Vet Intern Med* 1990;4:247–253.
- Kargman S, Charleson S, Cartwright M, Frank J, et al. Characterization of prostaglandin G/H synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. *Gastroenterology* 1996;111:445–454.

- Karmeli F, Cohen P, Rachmilewitz D. Cyclo-oxygenase-2 inhibitors ameliorate the severity of experimental colitis in rats. *Eur J Gastroenterol Hepatol* 2000;12:223–231.
- Kataoka H, Horie Y, Koyama R, Nakatsugi S, et al. Interaction between NSAIDs and steroid in rat stomach: safety of nimesulide as a preferential COX-2 inhibitor in the stomach. *Dig Dis Sci* 2000;45:1366–1375.
- Kato S, Ogawa Y, Kanatsu K, Okayama M, et al. Ulcerogenic influence of selective cyclooxygenase-2 inhibitors in the rat stomach with adjuvant-induced arthritis. *J Pharmacol Exp Ther* 2002;303:503–509.
- Kato S, Ito Y, Nishio H, Aoi Y, et al. Increased susceptibility of small intestine to NSAID- provoked ulceration in rats with adjuvant-induced arthritis: involvement of enhanced expression of TLR4. *Life Sci* 2007;81:1309–1316.
- Katz LB, Genna T, Fuller BL, Tolman EL, et al. Altered susceptibility of arthritic rats to the gastric lesion-inducing effects of aspirin or ethanol and the antilesion effect of rioprostil. *Agents Actions* 1987;22:134–143.
- Kauffman G. Aspirin-induced gastric mucosal injury: lessons learned from animal models. *Gastroenterology* 1989;96:606–614.
- Kearney D, Byrne A, Crean P, Cox D, et al. Optimal suppression of thromboxane A(2) formation by aspirin during percutaneous transluminal coronary angioplasty: no additional effect of a selective cyclooxygenase-2 inhibitor. *J Am Coll Cardiol* 2004;43:526–531.
- Kent TH, Cardelli RM, Stamler FW. Small intestinal ulcers and intestinal flora in rats given indomethacin. *Am J Pathol* 1969;54:237–249.
- Khan KN, Paulson S, Seibert K, Maziasz T. Gastrointestinal and renal safety of a specific COX-2 inhibitor versus NSAIDs in dogs. *Vet Pathol* 1997;34:509.
- Khan KN, Masferrer JL, Woerner BM, Soslow R, et al. Enhanced cyclooxygenase-2 expression in sporadic and familial adenomatous polyposis of the human colon. *Scand J Gastroenterol* 2001;36:865–869.
- Kim SW, Parekh D, Townsend CM Jr, Thompson JC. Effects of aging on duodenal bicarbonate secretion. *Ann Surg* 1990;212:332–337.
- King JN, Dawson J, Esser RE, Fujimoto R, et al. Preclinical pharmacology of robenacoxib: a novel selective inhibitor of cyclooxygenase-2. *J Vet Pharmacol Ther* 2009;32:1–17.
- Kishimoto Y, Wada K, Nakamoto K, Kawasaki H, et al. Levels of cyclooxygenase-1 and -2 mRNA expression at various stages of acute gastric injury induced by ischemia-reperfusion in rats. *Arch Biochem Biophys* 1998;352:153–157.
- Knapp DW, Richardson RC, Bottoms GD, Teclaw R, et al. Phase I trial of piroxicam in 62 dogs bearing naturally occurring tumors. *Cancer Chemother Pharmacol* 1992;29:214–218.
- Knapp DW, Richardson RC, Chan TC, Bottoms GD, et al. Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. *J Vet Intern Med* 1994;8:273–278.
- Koki A, Khan NK, Woerner BM, Dannenberg AJ, et al. Cyclooxygenase-2 in human pathological disease. *Adv Exp Med Biol* 2002a;507:177–184.
- Koki AT, Khan NK, Woerner BM, Seibert K, et al. Characterization of cyclooxygenase-2 (COX-2) during tumorigenesis in human epithelial cancers: evidence for potential clinical utility of COX-2 inhibitors in epithelial cancers. *Prostaglandins Leukot Essent Fatty Acids* 2002b;66:13–18.
- Kopcha M, Ahl AS. Experimental uses of flunixin meglumine and phenylbutazone in food-producing animals. *J Am Vet Med Assoc* 1989;19:445–449.
- Korytko PJ, Senese PB, Sellers RS, Cai W, et al. Gastrointestinal and renal toxicity associated with loxoprofen sodium in dogs. *Preclinica* 2003;1:107–113.
- Krause MM, Brand MD, Krauss S, Meisel C, et al. Nonsteroidal antiinflammatory drugs and a selective cyclooxygenase 2 inhibitor uncouple mitochondria in intact cells. *Arthritis Rheum* 2003;48:1438–1444.
- La Corte R, Caselli M, Castellino G, Bajocchi G, et al. Prophylaxis and treatment of NSAID-induced gastroduodenal disorders. *Drug Saf* 1999;20:527–543.
- Laine L, Sloane R, Ferretti M, Cominelli F. A randomized double blind comparison of placebo, etodolac, and naproxen on gastrointestinal injury and prostaglandin production. *Gastrointest Endosc* 1995;42:428–433.

- Laine L, Harper S, Simon T, Bath R, et al. A randomized trial comparing the effect of rofecoxib, a cyclooxygenase 2-specific inhibitor, with that of ibuprofen on the gastroduodenal mucosa of patients with osteoarthritis. Rofecoxib Osteoarthritis Endoscopy Study Group. *Gastroenterology* 1999;117:776–783.
- Laine L, Curtis SP, Cryer B, Kaur A, et al. Assessment of upper gastrointestinal safety of etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-Term (MEDAL) programme: a randomised comparison. *Lancet* 2007;369:465–473.
- Lamon TK, Browder EJ, Sohrabji F, Ihrig M. Adverse effects of incorporating ketoprofen into established rodent studies. *J Am Assoc Lab Anim Sci* 2008;47:20–24.
- Lanas A, Panes J, Pique JM. Clinical implications of COX-1 and/or COX-2 inhibition for the distal gastrointestinal tract. *Curr Pharm Des* 2003;9:2253–2266.
- Langenbach R, Morham SG, Tiano HF, Loftin CD, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 1995;83:483–492.
- Langman MJ, Jensen DM, Watson DJ, Harper SE, et al. Adverse upper gastrointestinal effects of rofecoxib compared with NSAIDs. *J Am Med Assoc* 1999;282:1929–1933.
- Langston C, Clarke CR. The role of the clinical pharmacologist in animal health. *AAPS PharmSci* 2002;4:E36.
- Lanza FL, Nelson RS, Greenberg BP. Effects of fenbufen, indomethacin, naproxen, and placebo on gastric mucosa of normal volunteers: a comparative endoscopic and photographic evaluation. *Am J Med* 1983;75:75–79.
- Lanza FL, Marathi UK, Anand BS, Lichtenberger LM. Clinical trial: comparison of ibuprofen-phosphatidylcholine and ibuprofen on the gastrointestinal safety and analgesic efficacy in osteoarthritic patients. *Aliment Pharmacol Ther* 2008;28:431–442.
- Lascelles BD, Bliklager AT, Fox SM, Reece D. Gastrointestinal tract perforation in dogs treated with a selective cyclooxygenase-2 inhibitor: 29 cases (2002–2003). *J Am Vet Med Assoc* 2005a;227:1112–1117.
- Lascelles BD, McFarland JM, Swann H. Guidelines for safe and effective use of NSAIDs in dogs. *Vet Ther* 2005b;6:237–251.
- Laudanno OM, Cesolari JA, Esnarriaga J, Rista L, et al. Gastrointestinal damage induced by celecoxib and rofecoxib in rats. *Dig Dis Sci* 2001;46:779–784.
- Lazzaroni M, Porro GB. Management of NSAID-induced gastrointestinal toxicity: focus on proton pump inhibitors. *Drugs* 2009;69:51–69.
- Lee M, Feldman M. Age-related reductions in gastric mucosal prostaglandin levels increase susceptibility to aspirin-induced injury in rats. *Gastroenterology* 1994;107:1746–1750.
- Lee, M. Age-related changes in gastric mucosal bicarbonate secretion in rats. *Age* 1996;19:55–58.
- Leedham SJ, Brittan M, Preston SL, McDonald SA, et al. The stomach periglandular fibroblast sheath: all present and correct. *Gut* 2006;55:295–296.
- Lees P, Higgins AJ. Clinical pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs on the horse. *Equine Vet J* 1985;17:83–96.
- Lees P, Landoni MF, Giraudel J, Toutain PL. Pharmacodynamics and pharmacokinetics of non-steroidal anti-inflammatory drugs in species of veterinary interest. *J Vet Pharmacol Ther* 2004;27:479–490.
- Legen I, Kristl A. pH and energy dependent transport of ketoprofen across rat jejunum in vitro. *Eur J Pharm Biopharm* 2003;56:87–94.
- Leite S, Martins NM, Dorta DJ, Curti C, et al. Mitochondrial uncoupling by the sulindac metabolite, sulindac sulfide. *Basic Clin Pharmacol Toxicol* 2006;99:294–299.
- Le Loet X. Safety of ketoprofen in the elderly: a prospective study on 20,000 patients. *Scand J Rheumatol Suppl* 1989;83:21–27.
- Lesch CA, Kraus ER, Sanchez B, Gilbertsen R, et al. Lack of beneficial effect of COX-2 inhibitors in an experimental model of colitis. *Methods Find Exp Clin Pharmacol* 1999;21:99–104.
- Li JT, Li ZS, Zhan XB, Cui ZM, et al. Experimental study of the safety of the selective COX-2 inhibitor, celecoxib, for gastric mucosa. *Chin J Dig Dis* 2003;4:53–56.

- Linden DR, Sharkey KA, Ho W, Mawe GM. Cyclooxygenase-2 contributes to dysmotility and enhanced excitability of myenteric AH neurones in the inflamed guinea pig distal colon. *J Physiol* 2004;557:191–205.
- Lipowitz AJ, Boulay JP, Klausner JS. Serum salicylate concentrations and endoscopic evaluation of the gastric mucosa in dogs after oral administration of aspirin-containing products. *Am J Vet Res* 1986;47:1586–1589.
- Lipscomb GR, Wallis N, Armstrong G, Rees WD. Gastrointestinal tolerability of meloxicam and piroxicam: a double-blind placebo-controlled study. *Br J Clin Pharmacol* 1998;46:133–137.
- Lisse JR, Perlman M, Johansson G, Shoemaker JR, et al. Gastrointestinal tolerability and effectiveness of rofecoxib versus naproxen in the treatment of osteoarthritis: a randomized, controlled trial. *Ann Intern Med* 2003;139:539–546.
- Lohmander LS, McKeith D, Svensson O, Malmenäs M, et al. A randomised, placebo controlled, comparative trial of the gastrointestinal safety and efficacy of AZD3582 versus naproxen in osteoarthritis. *Ann Rheum Dis* 2005;64:449–456.
- Luna SP, Basílio AC, Steagall PV, Machado LP, et al. Evaluation of adverse effects of long-term oral administration of carprofen, etodolac, flunixin meglumine, ketoprofen, and meloxicam in dogs. *Am J Vet Res* 2007;68:258–264.
- MacAllister CG. Effects of toxic doses of phenylbutazone in ponies. *Am J Vet Res* 1983;44:2277–2279.
- MacAllister CG, Morgan SJ, Borne AT, Pollet RA. Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *J Am Vet Med Assoc* 1993;202:71–77.
- Mackay RJ, French TW, Nguyen HT, Mayhew IG. Effects of large doses of phenylbutazone administration to horses. *Am J Vet Res* 1983;44:774–780.
- Magdalou J, Chajes V, Lafaurie C, Siest G. Glucuronidation of 2-arylpropionic acids pirofen, flurbiprofen, and ibuprofen by liver microsomes. *Drug Metab Dispos* 1990;18:692–697.
- Mahmud T, Rafi SS, Scott DL, Wrigglesworth JM, et al. Nonsteroidal antiinflammatory drugs and uncoupling of mitochondrial oxidative phosphorylation. *Arthritis Rheum* 1996;39:1998–2003.
- Martín AR, Villegas I, Alarcón de la Lastra C. The COX-2 inhibitor, rofecoxib, ameliorates dextran sulphate sodium induced colitis in mice. *Inflamm Res* 2005;54:145–151.
- Martínez C, Blanco G, Ladero JM, García-Martín E, et al. Genetic predisposition to acute gastrointestinal bleeding after NSAIDs use. *Br J Pharmacol* 2004;141:205–208.
- Masferrer JL, Zweifel BS, Manning PT, Hauser SD, et al. Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. *Proc Natl Acad Sci USA* 1994;91:3228–3232.
- Mason JC. NSAIDs and the oesophagus. *Eur J Gastroenterol Hepatol* 1999;11:369–373.
- Mathews KA. Nonsteroidal anti-inflammatory analgesics in pain management in dogs and cats. *Can Vet J* 1996;37:539–545.
- Maziasz TJ, Khan KNM, Talley J, Gierse J, et al. The development of drugs that target cyclooxygenase-2. In: Harris RE (ed), *Cyclooxygenase 2 Blockade in Cancer Prevention and Therapy*. Clifton, UK: Humana Press, 2003, pp. 259–277.
- McCafferty DM, Granger DN, Wallace JL. Indomethacin induced gastric injury and leukocyte adherence in arthritic versus healthy rats. *Gastroenterology* 1995;109:1173–1180.
- McConnell EL, Basit AW, Murdan S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. *J Pharm Pharmacol* 2008;60:63–70.
- McConnico RS, Morgan TW, Williams CC, Hubert JD, et al. Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses. *Am J Vet Res* 2008;69:1496–1505.
- Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 1998;2:253–258.
- Melorange R, Gentry C, O'Connell C, Blower PR, et al. Anti-inflammatory and gastrointestinal effects of nabumetone or its active metabolite, 6MNA (6-methoxy-2-naphthylacetic acid): comparison with indomethacin. *Agents Actions* 1992;Spec No:C82–C83.
- Menezes GB, Rezende RM, Pereira-Silva PE, Klein A, et al. Differential involvement of cyclooxygenase isoforms in neutrophil migration in vivo and in vitro. *Eur J Pharmacol* 2008;598:118–122.
- Menozi A, Pozzoli C, Poli E, Dacasto M, et al. Effects of nonselective and selective cyclooxygenase inhibitors on small intestinal motility in the horse. *Res Vet Sci* 2009;86:129–135.
- Mersereau WA, Hinchey EJ. Synergism between acid and gastric contractile activity in the genesis of ulceration and hemorrhage in the phenylbutazone-treated rat. *Surgery* 1981;90:516–522.

- Meschter CL, Gilbert M, Krook L, Maylin G, et al. The effects of phenylbutazone on the intestinal mucosa of the horse: a morphological, ultrastructural and biochemical study. *Equine Vet J* 1990a;22:255–263.
- Meschter CL, Gilbert M, Krook L, Maylin G, et al. The effects of phenylbutazone on the morphology and prostaglandin concentrations of the pyloric mucosa of the equine stomach. *Vet Pathol* 1990b;27:244–253.
- Moberly JB, Harris SI, Riff DS, Dale JC, et al. A randomized, double-blind, one-week study comparing effects of a novel COX-2 inhibitor and naproxen on the gastric mucosa. *Dig Dis Sci* 2007;52:442–450.
- Modlin IM, Kidd M, Lye KD, Wright NA. Gastric stem cells: an update. *Keio J Med* 2003;52:134–137.
- Möllenhoff A, Nolte I, Kramer S. Anti-nociceptive efficacy of carprofen, levomethadone and buprenorphine for pain relief in cats following major orthopaedic surgery. *J Vet Med A Physiol Pathol Clin Med* 2005;52:186–198.
- Moore JN, Garner HE, Shapland JE, Hatfield DG. Prevention of endotoxin-induced arterial hypoxaemia and lactic acidosis with flunixin meglumine in the conscious pony. *Equine Vet J* 1981;13:95–98.
- Morham SG, Langenbach R, Loftin CD, Tiano HF, et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* 1995;83:473–482.
- Moses T, Wagner L, Fleming SD. TLR4-mediated COX-2 expression increases intestinal ischemia/reperfusion-induced damage. *J Leukoc Biol* 2009;86:971–980.
- Murtaugh RJ, Matz ME, Labato MA, Boudrieau RJ. Use of synthetic prostaglandin E1 (misoprostol) for prevention of aspirin-induced gastroduodenal ulceration in arthritic dogs. *J Am Vet Med Assoc* 1993;202:251–256.
- Naito Y, Yoshikawa T. Oxidative stress involvement and gene expression in indomethacin-induced gastropathy. *Redox Rep* 2006;11:243–253.
- Nandi J, Das PK, Zinkievich JM, Baltodano JD, et al. Cyclo-oxygenase-1 inhibition increases acid secretion by modulating H⁺, K⁺-ATPase expression and activation in rabbit parietal cells. *Clin Exp Pharmacol Physiol* 2009;36:127–134.
- Nap RC, Breen DJ, Lam TJ, De Bruyne JJ. Gastric retention of enteric-coated aspirin tablets in beagle dogs. *J Vet Pharmacol Ther* 1990;13:148–153.
- Narita T, Sato R, Tomizawa N, Tani K, et al. Safety of reduced-dosage ketoprofen for long-term oral administration in healthy dogs. *Am J Vet Res* 2006;67:1115–1120.
- Neuss H, Raue W, Müller V, Schwenk W, et al. Effects of cyclooxygenase inhibition on anastomotic healing following large bowel resection in a rabbit model: a randomized, blinded, placebo-controlled trial. *Int J Colorectal Dis* 2009;24:551–557.
- Nguyen T, Chai J, Li A, Akahoshi T, et al. Novel roles of local insulin-like growth factor-1 activation in gastric ulcer healing: promotes actin polymerization, cell proliferation, re-epithelialization, and induces cyclooxygenase-2 in a phosphatidylinositol 3-kinase-dependent manner. *Am J Pathol* 2007;170:1219–1228.
- NygAard G, Anthony A, Khan K, Bounds SV, et al. Intestinal site-dependent susceptibility to chronic indomethacin in the rat: a morphological and biochemical study. *Aliment Pharmacol Ther* 1995;9:403–410.
- Oberto G, Conz A, Giachetti C, Galli CL. Evaluation of the toxicity of indomethacin in a 4-week study, by oral route, in the marmoset (*Callithrix jacchus*). *Fundam Appl Toxicol* 1990;15:800–813.
- Ofman JJ, Maclean CH, Straus WL, Morton SC, et al. Meta-analysis of dyspepsia and nonsteroidal antiinflammatory drugs. *Arthritis Rheum* 2003;49:508–518.
- Okayama M, Hayashi S, Aoi Y, Nishio H, et al. Aggravation by selective COX-1 and COX-2 inhibitors of dextran sulfate sodium (DSS)-induced colon lesions in rats. *Dig Dis Sci* 2007;52:2095–2103.
- Pai R, Soreghan B, Szabó IL, Pavelka M, et al. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* 2002;8:289–293.
- Patoia L, Santucci L, Furno P, Dionisi MS, et al. A 4-week, double-blind, parallel-group study to compare the gastrointestinal effects of meloxicam 7.5mg, meloxicam 15mg, piroxicam 20mg and placebo by means of faecal blood loss, endoscopy and symptom evaluation in healthy volunteers. *Br J Rheumatol* 1996;35 Suppl 1: 61–67.

- Patrono C, Patrignani P, García Rodríguez LA. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. *J Clin Invest* 2001;108:7–13.
- Pavelka K, Recker DP, Verburg KM. Valdecoxib is as effective as diclofenac in the management of rheumatoid arthritis with a lower incidence of gastroduodenal ulcers: results of a 26-week trial. *Rheumatology (Oxford)* 2003;42:1207–1215.
- Petrescu I, Tarba C. Uncoupling effects of diclofenac and aspirin in the perfused liver and isolated hepatic mitochondria of rat. *Biochim Biophys Acta* 1997;1318:385–394.
- Pihl L, Nylander O. Products of cyclooxygenase-2 depress duodenal function in rats subjected to abdominal surgery. *Acta Physiol (Oxford)* 2006;186:279–290.
- Pilotto A, Seripa D, Franceschi M, Scarcelli C, et al. Genetic susceptibility to nonsteroidal anti-inflammatory drug-related gastroduodenal bleeding: role of cytochrome P450 2C9 polymorphisms. *Gastroenterology* 2007;133:465–471.
- Pollmeier M, Toulemonde C, Fleishman C, Hanson PD. Clinical evaluation of firocoxib and carprofen for the treatment of dogs with osteoarthritis. *Vet Rec* 2006;159:547–451.
- Poortinga EW, Hungerford LL. A case–control study of acute ibuprofen toxicity in dogs. *Prev Vet Med* 1998;35:115–124.
- Radi ZA. Pathophysiology of cyclooxygenase inhibition in animal models. *Toxicol Pathol* 2009;37:34–46.
- Radi ZA, Khan NK. Comparative expression and distribution of c-fos, estrogen receptor alpha (eralpha), and p38 alpha in the uterus of rats, monkeys, and humans. *Toxicol Pathol* 2006a;34:327–335.
- Radi ZA, Khan NK. Effects of cyclooxygenase inhibition on the gastrointestinal tract. *Exp Toxicol Pathol* 2006b;58:163–173.
- Radi ZA, Kehrl ME Jr, Ackermann MR. Cell adhesion molecules, leukocyte trafficking, and strategies to reduce leukocyte infiltration. *J Vet Intern Med* 2001;15:516–529.
- Radi ZA, Marusak RA, Morris DL. Species comparison of the role of p38 MAP kinase in the female reproductive system. *J Toxicol Pathol* 2009;22:109–124.
- Radi ZA, Heuvelman DM, Masferrer JL, Benson EL. Pharmacologic evaluation of sulfasalazine, FTY720, and anti-IL-12/23p40 in a TNBS-induced Crohn's disease model. *Dig Dis Sci* 2011;56:2283–2291.
- Raekallio MR, Hielm-Björkman AK, Kejonen J, Salonen HM, et al. Evaluation of adverse effects of long-term orally administered carprofen in dogs. *J Am Vet Med Assoc* 2006;228:876–880.
- Rainsford KD. The comparative gastric ulcerogenic activities of non-steroid anti-inflammatory drugs. *Agents Actions* 1977;7:573–577.
- Rainsford KD. An analysis of the gastro-intestinal side-effects of non-steroidal anti-inflammatory drugs, with particular reference to comparative studies in man and laboratory species. *Rheumatol Int* 1982;2:1–10.
- Rainsford KD. Ibuprofen: pharmacology, efficacy and safety. *Inflammopharmacology* 2009;17:275–342.
- Rainsford KD, Stetsko PI, Sirko SP, Debski S. Gastrointestinal mucosal injury following repeated daily oral administration of conventional formulations of indometacin and other non-steroidal anti-inflammatory drugs to pigs: a model for human gastrointestinal disease. *J Pharm Pharmacol* 2003;55:661–668.
- Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, et al. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004;118:229–241.
- Rampal P, Moore N, Van Ganse E, Le Parc JM, et al. Gastrointestinal tolerability of ibuprofen compared with paracetamol and aspirin at over-the-counter doses. *J Int Med Res* 2002;30:301–308.
- Raveendran NN, Silver K, Freeman LC, Narvaez D, et al. Drug-induced alterations to gene and protein expression in intestinal epithelial cell 6 cells suggest a role for calpains in the gastrointestinal toxicity of nonsteroidal anti-inflammatory agents. *J Pharmacol Exp Ther* 2008;325:389–399.
- Reed S. Nonsteroidal anti-inflammatory drug-induced duodenal ulceration and perforation in a mature rottweiler. *Can Vet J* 2002;43:971–972.
- Reimer ME, Johnston SA, Leib MS, Duncan RB, et al. The gastroduodenal effects of buffered aspirin, carprofen, and etodolac in healthy dogs. *J Vet Intern Med* 1999;13:472–477.
- Reuter BK, Davies NM, Wallace JL. Nonsteroidal anti-inflammatory drug enteropathy in rats: role of permeability, bacteria, and enterohepatic circulation. *Gastroenterology* 1997;112:109–117.

- Ricketts AP, Lundy KM, Seibel SB. Evaluation of selective inhibition of canine cyclooxygenase 1 and 2 by carprofen and other nonsteroidal anti-inflammatory drugs. *Am J Vet Res* 1998;59:1441–1446.
- Roberts ES, Van Lare KA, Marable BR, Salminen WF. Safety and tolerability of 3-week and 6-month dosing of Deramaxx (deracoxib) chewable tablets in dogs. *J Vet Pharmacol Ther* 2009;32:329–337.
- Roberts PJ, Morgan K, Miller R, Hunter JO, et al. Neuronal COX-2 expression in human myenteric plexus in active inflammatory bowel disease. *Gut* 2001;48:468–472.
- Robinson MG. New oral salicylates in the therapy of chronic idiopathic inflammatory bowel disease. *Gastroenterol Clin North Am* 1989;18:43–50.
- Rostom A, Muir K, DuBe C, Jolicoeur E, et al. Gastrointestinal safety of cyclooxygenase-2 inhibitors: a Cochrane Collaboration systematic review. *Clin Gastroenterol Hepatol* 2007;5:818–828.
- Roudebush P, Morse GE. Naproxen toxicosis in a dog. *J Am Vet Med Assoc* 1981;179:805–806.
- Rubio F, Seawall S, Pocolinko R, DeBarbieri B, et al. Metabolism of carprofen, a nonsteroid anti-inflammatory agent, in rats, dogs, and humans. *J Pharm Sci* 1980;69:1245–1253.
- Runkel R, Chaplin M, Boost G, Segre E, et al. Absorption, distribution, metabolism, and excretion of naproxen in various laboratory animals and human subjects. *J Pharm Sci* 1972;61:703–708.
- Ryan WG, Moldave K, Carithers D. Clinical effectiveness and safety of a new NSAID, firocoxib: a 1,000 dog study. *Vet Ther* 2006;7:119–126.
- Sato N, Kozar RA, Zou L, Weatherall JM, et al. Peroxisome proliferator-activated receptor gamma mediates protection against cyclooxygenase-2-induced gut dysfunction in a rodent model of mesenteric ischemia/reperfusion. *Shock* 2005;24:462–469.
- Sawant S, Snyman C, Bhoola K. Comparison of tissue kallikrein and kinin receptor expression in gastric ulcers and neoplasms. *Int Immunopharmacol* 2001;1:2063–2080.
- Sawaoka H, Tsuji S, Tsuji M, Gunawan ES, et al. Expression of the cyclooxygenase-2 gene in gastric epithelium. *J Clin Gastroenterol* 1997; S105–S110.
- Sawaoka H, Tsuji S, Tsuji M, Gunawan ES, et al. Involvement of cyclooxygenase-2 in proliferation and morphogenesis induced by transforming growth factor alpha in gastric epithelial cells. *Prostaglandins Leukot Essent Fatty Acids* 1999;61:315–322.
- Schattenkirchner M. Long-term safety of ketoprofen in an elderly population of arthritic patients. *Scand J Rheumatol Suppl* 1991;91:27–36.
- Scheiman JM. NSAIDs, gastrointestinal injury, and cytoprotection. *Gastroenterol Clin North Am* 1996;25:279–298.
- Scheiman JM. Gastrointestinal safety of cyclooxygenase-2 inhibitors. *Curr Pharm Des* 2003;9:2197–2206.
- Scheiman JM, Greenson JK, Lee J, Cryer B. Effect of cyclooxygenase-2 inhibition on human *Helicobacter pylori* gastritis: mechanisms underlying gastrointestinal safety and implications for cancer chemoprevention. *Aliment Pharmacol Ther* 2003;17:1535–1543.
- Scherkl R, Frey HH. Pharmacokinetics of ibuprofen in the dog. *J Vet Pharmacol Ther* 1987;10:261–265.
- Scherle PA, Ma W, Lim H, Dey SK, et al. Regulation of cyclooxygenase-2 induction in the mouse uterus during decidualization: an event of early pregnancy. *J Biol Chem* 2000;275:37086–37092.
- Schmassmann A, Peskar BM, Stettler C, Netzer P, et al. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. *Br J Pharmacol* 1998;123:795–804.
- Schnitzer TJ, Burmester GR, Mysler E, Hochberg MC, et al. Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), reduction in ulcer complications: randomised controlled trial. *Lancet* 2004;364:665–674.
- Schoen RT, Vender RJ. Mechanisms of nonsteroidal anti-inflammatory drug-induced gastric damage. *Am J Med* 1989;86:449–458.
- Schoenfeld P, Kimmey MB, Scheiman J, Bjorkman D, et al. Review article: nonsteroidal anti-inflammatory drug-associated gastrointestinal complications—guidelines for prevention and treatment. *Aliment Pharmacol Ther* 1999;13:1273–1285.
- Schrivver DA, White CB, Sandor A, Rosenthale ME. A profile of the rat gastrointestinal toxicity of drugs used to treat inflammatory diseases. *Toxicol Appl Pharmacol* 1975;32:73–83.
- Schwarz NT, Kalf J, Türler A, Engel BM, et al. Prostanoid production via COX-2 as a causative mechanism of rodent postoperative ileus. *Gastroenterology* 2001;121:1354–1371.
- Seibert K, Zhang Y, Leahy K, Hauser S, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci USA* 1994;91:12013–12017.

- Seibert K, Zhang Y, Leahy K, Hauser S, et al. Distribution of COX-1 and COX-2 in normal and inflamed tissues. *Adv Exp Med Biol* 1997;400A:167–170.
- Seitz S, Boelsterli UA. Diclofenac acyl glucuronide, a major biliary metabolite, is directly involved in small intestinal injury in rats. *Gastroenterology* 1998;115:1476–1482.
- Seitz S, Kretz-Rommel A, Oude Elferink RP J, Boelsterli UA. Selective protein adduct formation of diclofenac glucuronide is critically dependent on the rat canalicular conjugate export pump (Mrp2). *Chem Res Toxicol* 1998;11:513–519.
- Sennello KA, Leib MS. Effects of deracoxib or buffered aspirin on the gastric mucosa of healthy dogs. *J Vet Intern Med* 2006;20:1291–1296.
- Shifflett DE, Jones SL, Moeser AJ, Blikslager AT. Mitogen-activated protein kinases regulate COX-2 and mucosal recovery in ischemic-injured porcine ileum. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G906–G913.
- Shriver DA, Dove PA, White CB, Sandor A, et al. A profile of the gastrointestinal toxicity of aspirin, indomethacin, oxaprozin, phenylbutazone, and fentiazac in arthritic and Lewis normal rats. *Toxicol Appl Pharmacol* 1977;42:75–83.
- Sigthorsson G, Jacob M, Wrigglesworth J, Somasundaram S, et al. Comparison of indomethacin and nimesulide, a selective cyclooxygenase-2 inhibitor, on key pathophysiologic steps in the pathogenesis of nonsteroidal anti-inflammatory drug enteropathy in the rat. *Scand J Gastroenterol* 1998;33:728–735.
- Sigthorsson G, Simpson RJ, Walley M, Anthony A, et al. COX-1 and 2, intestinal integrity, and pathogenesis of nonsteroidal anti-inflammatory drug enteropathy in mice. *Gastroenterology* 2002;122:1913–1923.
- Silverstein FE, Faich G, Goldstein JL, Simon LS, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study—a randomized controlled trial. Celecoxib Long-Term Arthritis Safety Study. *J Am Med Assoc* 2000;284:1247–1255.
- Singer II, Kawka DW, Schloemann S, Tessner T, et al. Cyclooxygenase 2 is induced in colonic epithelial cells in inflammatory bowel disease. *Gastroenterology* 1998;115:297–306.
- Singh G, Fort JG, Goldstein JL, Levy RA, et al. Celecoxib versus naproxen and diclofenac in osteoarthritis patients: SUCCESS-I Study. *Am J Med* 2006;119:255–266.
- Singh VP, Patil CS, Jain NK, Kulkarni SK. Aggravation of inflammatory bowel disease by cyclooxygenase-2 inhibitors in rats. *Pharmacology* 2004;72:77–84.
- Siraki AG, Chevalдина T, O'Brien PJ. Application of quantitative structure–toxicity relationships for acute NSAID cytotoxicity in rat hepatocytes. *Chem Biol Interact* 2005;151:177–191.
- Smith HW. Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J Pathol Bacteriol* 1965;89:95–122.
- Somasundaram S, Sigthorsson G, Simpson RJ, Watts J, et al. Uncoupling of intestinal mitochondrial oxidative phosphorylation and inhibition of cyclooxygenase are required for the development of NSAID-enteropathy in the rat. *Aliment Pharmacol Ther* 2000;14:639–650.
- Soslow RA, Dannenberg AJ, Rush D, Woerner BM, et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. *Cancer* 2000;89:2637–2645.
- Spangler RS. Gastrointestinal damage demonstrated with nabumetone or etodolac in preclinical studies. *Am J Med* 1993;95:35S–39S.
- Stanton ME, Bright RM. Gastroduodenal ulceration in dogs: retrospective study of 43 cases and literature review. *J Vet Intern Med* 1989;3:238–244.
- Steagall PV, Mantovani FB, Ferreira TH, Salcedo ES, et al. Evaluation of the adverse effects of oral firocoxib in healthy dogs. *J Vet Pharmacol Ther* 2008;30:218–223.
- Steagall PV, Moutinho FQ, Mantovani FB, Passarelli D, et al. Evaluation of the adverse effects of subcutaneous carprofen over six days in healthy cats. *Res Vet Sci* 2009;86:115–120.
- Stoltz RR, Harris SI, Kuss ME, LeComte D, et al. Upper GI mucosal effects of parecoxib sodium in healthy elderly subjects. *Am J Gastroenterol* 2002;97:65–71.
- Sturm A, Dignass AU. Epithelial restitution and wound healing in inflammatory bowel disease. *World J Gastroenterol* 2008;14:348–353.
- Sun WH, Tsuji S, Tsuji M, Gunawan ES, et al. Cyclo-oxygenase-2 inhibitors suppress epithelial cell kinetics and delay gastric wound healing in rats. *J Gastroenterol Hepatol* 2000;15:752–761.

- Szabó IL, Pai R, Jones MK, Ehring GR, et al. Indomethacin delays gastric restitution: association with the inhibition of focal adhesion kinase and tensin phosphorylation and reduced actin stress fibers. *Exp Biol Med (Maywood)* 2002;227:412–424.
- Takeuchi K, Okada M, Ebara S, Osano H. Increased microvascular permeability and lesion formation during gastric hypermotility caused by indomethacin and 2-deoxy-D-glucose in the rat. *J Clin Gastroenterol* 1990;12 Suppl 1:S76–S84.
- Takeuchi K, Ukawa H, Furukawa O, Kawauchi S, et al. Prostaglandin E receptor subtypes involved in stimulation of gastroduodenal bicarbonate secretion in rats and mice. *J Physiol Pharmacol* 1999;50:155–167.
- Takeuchi K, Miyazawa T, Tanaka A, Kato S, et al. Pathogenic importance of intestinal hypermotility in NSAID-induced small intestinal damage in rats. *Digestion* 2002;66:340–341.
- Takeuchi K, Tanaka A, Hayashi Y, Kubo Y. Functional mechanism underlying COX-2 expression following administration of indomethacin in rat stomachs: importance of gastric hypermotility. *Dig Dis Sci* 2004;49:180–187.
- Takeuchi K, Aihara E, Sasaki Y, Normura Y, et al. Involvement of cyclooxygenase-1, prostaglandin E2 and EP1 receptors in acid-induced HCO₃-secretion in stomach. *J Physiol Pharmacol* 2006;57:661–676.
- Takeuchi K, Tanaka A, Kato S, Amagase K, et al. Roles of COX inhibition in pathogenesis of NSAID-induced small intestinal damage. *Clin Chim Acta* 2010;411:459–466.
- Tanaka A, Araki H, Komoike Y, Hase S, et al. Inhibition of both COX-1 and COX-2 is required for development of gastric damage in response to nonsteroidal anti-inflammatory drugs. *J Physiol Paris* 2001;95:21–27.
- Tanaka A, Araki H, Hase S, Komoike Y, et al. Up-regulation of COX-2 by inhibition of COX-1 in the rat: a key to NSAID-induced gastric injury. *Aliment Pharmacol Ther* 2002a;16:90–101.
- Tanaka A, Hase S, Miyazawa T, Ohno R, et al. Role of cyclooxygenase (COX)-1 and COX-2 inhibition in nonsteroidal anti-inflammatory drug-induced intestinal damage in rats: relation to various pathogenic events. *J Pharmacol Exp Ther* 2002b;303:1248–1254.
- Tarnawski A, Stachura J, Durbin T, Sarfeh II, et al. Increased expression of epidermal growth factor receptor during gastric ulcer healing in rats. *Gastroenterology* 1992;102:695–698.
- Tatsuguchi A, Sakamoto C, Wada K, Akamatsu T, et al. Localisation of cyclooxygenase 1 and cyclooxygenase 2 in Helicobacter pylori-related gastritis and gastric ulcer tissues in humans. *Gut* 2000;46:782–789.
- Tibble JA, Sigthorsson G, Foster R, Bjarnason I. Comparison of the intestinal toxicity of celecoxib, a selective COX-2 inhibitor, and indomethacin in the experimental rat. *Scand J Gastroenterol* 2000;35:802–807.
- Tobin T, Chay S, Kamerling S, Woods WE, et al. Phenylbutazone in the horse: a review. *J Vet Pharmacol Ther* 1986;9:1–25.
- Tomlinson JE, Wilder BO, Young KM, Bliklager AT. Effects of flunixin meglumine or etodolac treatment on mucosal recovery of equine jejunum after ischemia. *Am J Vet Res* 2004;65:761–769.
- Traub JL, Gallina AM, Grant BD, Reed SM, et al. Phenylbutazone toxicosis in the foal. *Am J Vet Res* 1983;44:1410–1418.
- Traub-Dargatz JL, Bertone JJ, Gould DH, Wrigley RH, et al. Chronic flunixin meglumine therapy in foals. *Am J Vet Res* 1988;49:7–12.
- Tsubouchi R, Hayashi S, Aoi Y, Nishio H, et al. Healing impairment effect of cyclooxygenase inhibitors on dextran sulfate sodium-induced colitis in rats. *Digestion* 2006;74:91–100.
- Uchida M, Kawano O, Misaki N, Saitoh K, et al. Healing of acetic acid-induced gastric ulcer and gastric mucosal PGI₂ level in rats. *Dig Dis Sci* 1990;35:80–85.
- Ungaro R, Fukata M, Hsu D, Hernandez Y, et al. A novel Toll-like receptor 4 antagonist antibody ameliorates inflammation but impairs mucosal healing in murine colitis. *Am J Physiol Gastrointest Liver Physiol* 2009;296:G1167–G1179.
- Van Miert AS, Van Der Wal-Komproe LE, Van Duin CT. Effects of antipyretic agents on fever and ruminal stasis induced by endotoxins in conscious goats. *Arch Int Pharmacodyn Ther* 1977;225:39–50.
- Villar D, Buck WB, Gonzalez JM. Ibuprofen, aspirin and acetaminophen toxicosis and treatment in dogs and cats. *Vet Hum Toxicol* 1998;40:156–162.

- Villegas I, Alarcón de la Lastra C, Martín MJ, Motilva V, et al. Gastric damage induced by subchronic administration of preferential cyclooxygenase-1 and cyclooxygenase-2 inhibitors in rats. *Pharmacology* 2002;66:68–75.
- Vonderhaar MA, Salisbury SK. Gastroduodenal ulceration associated with flunixin meglumine administration in three dogs. *J Am Vet Med Assoc* 1993;203:92–95.
- Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am J Physiol* 1990;259:G462–G467.
- Wallace JL, McKnight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 2000;119:706–714.
- Watanabe T, Higuchi K, Kobata A, Nishio H, et al. Non-steroidal anti-inflammatory drug-induced small intestinal damage is Toll-like receptor 4 dependent. *Gut* 2008;57:181–187.
- Watanabe T, Nishio H, Tanigawa T, Yamagami H, et al. Probiotic *Lactobacillus casei* strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. *Am J Physiol Gastrointest Liver Physiol* 2009;297:G506–G513.
- Watson AD, Wilson JT, Turner DM, Culvenor JA. Phenylbutazone induced blood dyscrasias suspected in three dogs. *Vet Rec* 1980;107:239–241.
- Wax J, Clinger WA, Varner P, Bass P, et al. Relationship of the enterohepatic cycle to ulcerogenesis in the rat small bowel with flufenamic acid. *Gastroenterology* 1970;58:772–780.
- Wight NJ, Gottesdiener K, Garlick NM, Atherton CT, et al. Rofecoxib, a COX-2 inhibitor, does not inhibit human gastric mucosal prostaglandin production. *Gastroenterology* 2001;120:867–873.
- Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999;340:1888–1899.
- Wooten JG, Blikslager AT, Marks SL, Law JM, et al. Effect of nonsteroidal anti-inflammatory drugs with varied cyclooxygenase-2 selectivity on cyclooxygenase protein and prostanoid concentrations in pyloric and duodenal mucosa of dogs. *Am J Vet Res* 2009;70:1243–1249.
- Yesair DW, Callahan M, Remington L, Kensler CJ. Role of the entero-hepatic cycle of indomethacin on its metabolism, distribution in tissues and its excretion by rats, dogs and monkeys. *Biochem Pharmacol* 1970;19:1579–1590.
- Yokota A, Taniguchi M, Takahira Y, Tanaka A, et al. Rofecoxib produces intestinal but not gastric damage in the presence of a low dose of indomethacin in rats. *J Pharmacol Exp Ther* 2005;314:302–309.
- Yoshizawa T, Watanabe S, Hirose M, Yamamoto J, et al. Effects of growth factors on aspirin-induced inhibition of wound repair in a rabbit gastric epithelial cell model. *Aliment Pharmacol Ther* 2000;Suppl 1: 176–182.
- Zhang L, Lu YM, Dong XY. Effects and mechanism of the selective COX-2 inhibitor, celecoxib, on rat colitis induced by trinitrobenzene sulfonic acid. *Chin J Dig Dis* 2004;5:110–114.
- Zinkievich JM, George S, Jha S, Nandi J, et al. Gastric acid is the key modulator in the pathogenesis of NSAID-induced ulceration in rats. *Clin Exp Pharmacol Physiol* 2010;37:654–661.