NSAID-Induced Small Intestinal Damage – Roles of Various Pathogenic Factors

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**Abstract**

**Background/Aims:** NSAID-induced enteropathy has been the focus of recent basic and clinical research subsequent to the development of the capsule endoscope and double-balloon endoscope. We review the possible pathogenic mechanisms underlying NSAID-induced enteropathy and discuss the role of the inhibition of COX-1/COX-2 and the influences of food as well as various prophylactic treatments on these lesions. **Methods:** Studies were performed in experimental animals. **Results:** Multiple factors, such as intestinal hypermotility, decreased mucus secretion, enterobacteria, and upregulation of iNOS/NO expression, are involved in the pathogenesis of NSAID-induced enteropathy, in addition to the decreased production of PGs due to the inhibition of COX. Enterobacterial invasion is the most important pathogenic event, and intestinal hypermotility, which was associated with this event, is essential for the development of these lesions. NSAIDs also upregulate the expression of COX-2, and the inhibition of both COX-1 and COX-2 is required for the intestinal ulcerogenic properties of NSAIDs to manifest. NSAID-induced enteropathy is prevented by PGE\(_2\), atropine, ampicillin, and aminoguanidine as well as soluble dietary fiber, and exacerbated by antisecretory drugs such as proton pump inhibitors. **Conclusion:** These findings on the pathogenesis of NSAID-induced enteropathy will be useful for the future development of intestinal-sparing alternatives to standard NSAIDs. © 2015 S. Karger AG, Basel

**Key Words**

NSAID-induced enteropathy · Pathogenic mechanism · Prophylactic drugs · Dietary components · COX-1/COX-2 inhibition

**Introduction**

Damage to the upper gastrointestinal (GI) tract is a major adverse event associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin in experimental animals and humans [1, 2]. However, NSAID-induced small intestinal lesions have drawn particular attention recently as a result of the development of the capsule endoscope and double-balloon endoscope [3–6]. These techniques have enabled the identification of previously undetectable lesions in the human small intestine and demonstrated that such lesions are more common than previously thought.

Several factors are involved in the pathogenesis of NSAID-induced enteropathy, including a deficiency in prostaglandins (PGs), bile acid, bacterial flora, and nitric oxide (NO) [1, 7–11]; a deficiency in endogenous PGs has been identified as the most important factor for the occurrence of these lesions. The PG deficiency caused by NSAIDs has been attributed to the inhibition of cyclooxygenase (COX). COX exists in two isoforms; constitutively expressed COX-1 and inducible COX-2. The for-
mer is normally found in various tissues, including the small intestine, while the latter does not appear to be expressed, or at least at very low levels, in most tissues and is rapidly upregulated in response to growth factors and cytokines [12]. The ulcerogenic properties of NSAIDs in the GI tract are considered to be induced by the inhibition of COX-1, but not COX-2 [13]. However, recent studies showed that the inhibition of both COX-1 and COX-2 was required for NSAID-induced GI injury, suggesting a role of COX-2 as well as COX-1 in maintaining the mucosal integrity of these tissues [14–17].

At present, no satisfactory means for the prevention and treatment of these lesions in patients are available, except for the use of PG analogs; however, misoprostol, a PGE1 derivative, sometimes causes diarrhea and abdominal pain as side effects, which represent a significant impediment, especially for long-term treatment. Therefore, the identification of effective therapies for the treatment of NSAID-induced small intestinal lesions remains an urgent priority.

This chapter deals with NSAID-induced small bowel injuries, which have often been neglected in clinical practice as a potential cause of dyspeptic symptoms. Based on our publications, we reviewed the possible pathogenic mechanism underlying NSAID-induced intestinal damage and discussed the role of the inhibition of COX-1/COX-2; how they are related to the various pathogenic events of these lesions, including PG deficiency, intestinal motility, neutrophil infiltration, and NO production; and the influences of food as well as various prophylactic treatments on these lesions [8–10, 16, 18–21]. Since aspirin, unlike other conventional NSAIDs, does not cause damage in the small intestine after parenteral administration, despite inhibiting COX activity and the production of PG [22, 23], we also discussed the possible mechanism responsible for this phenomenon.

### Intestinal Ulcerogenic Action of NSAIDs

Conventional NSAIDs, such as indomethacin, diclofenac, flurbiprofen, and naproxen, produced hemorrhagic damage in the rat small intestine within 24 h, mainly in the jejunum and ileum [8, 16] (fig. 1a and b). These nonselective COX inhibitors at ulcerogenic doses caused a marked decrease in the mucosal PGE2 content of the small intestine. On the other hand, neither the selective COX-1 inhibitor SC-560 nor the selective COX-2 inhibitor rofecoxib caused any damage to the small intestine by themselves [16, 18] (fig. 1c). However, when these drugs were administered together, they induced hemorrhagic lesions in the small intestine at an incidence of 100%. Furthermore, when SC-560 was given together with increasing doses of rofecoxib, the severity of damage increased depending upon the dose of rofecoxib. Similar findings were obtained when rofecoxib was administered together with increasing doses of SC-560. Rofecoxib had no effect on the mucosal PGE2 content of the small intestine, while SC-560 at 10 mg/kg decreased the PGE2 content, with this effect being equivalent to that induced by indomethacin at 10 mg/kg. These findings argued the contention that COX-1, but not COX-2, plays a role in maintaining the mucosal integrity of the small intestine, and strongly suggest that the inhibition of both COX-1 and COX-2 is required for NSAID-induced intestinal ulceration.

Although clinical studies admitted a better GI safety profile of selective COX-2 inhibitors compared to conventional NSAIDs, they also showed that these drugs are not completely safe for the small bowel [17, 24–26]. Maidaen et al. [24] reported that long-term NSAIDs and COX-2-selective drugs caused comparable small-bowel damage, suggesting an important role for COX-2 in the maintenance of small-bowel integrity. Maehata et al. [25] also showed that the overall incidence of small-bowel mucosal injury was not different between the celecoxib group and the meloxicam group, although the mucosal lesion was less severe in the former. We previously reported that a selective COX-2 inhibitor alone damaged the gastric mucosa when the expression of COX-2 was upregulated in the stomach of rats subjected to adrenalectomy (glucocorticoid deficiency) or the induction of adjuvant arthritis [17, 27–29]. Under such conditions, the mucosal PGE2 contents were also shown to increase due to the upregulation of COX-2 expression [27, 28]. It is assumed that COX-2 played an important role in maintaining the integrity of the gastric mucosa in adrenalectomized or arthritic rats and that the selective COX-2 inhibitor by itself damaged the gastric mucosa by suppressing this additional PG production due to the up-regulation of COX-2 expression. Thus, the selective COX-2 inhibitor by itself may induce damage in the gastrointestinal tract of patients with arthritis or glucocorticoid deficiencies.

### Effects of Various Drugs on NSAID-Induced Small Intestinal Damage

Because no satisfactory means for the prophylaxis of NSAID-induced enteropathy in patients is currently available, the identification of effective drugs for the
treatment of these lesions is an urgent priority. Most of the drugs used in patients have been found to be effective in animal experiments, subsequently tested in clinical studies for their effects in patients. Therefore, the data in animal studies would give important and indispensable information for the development of therapeutic medicines.

The development of indomethacin-induced small intestinal lesions was previously shown to be prevented by supplementation with PGE₂ [9, 10, 16, 18, 19]. Ampicil-
lin, an antibiotic, also reduced the severity of intestinal damage in response to indomethacin. The severity of these lesions was also reduced by the selective iNOS inhibitor, aminoguanidine, or the anticholinergic drug, atropine [8, 19] (fig. 2a). NO interacts with superoxide radicals to produce a cytotoxic peroxynitrite, which has a deleterious influence on intestinal mucosal integrity [30]. Allopurinol, superoxide dismutase (SOD) or catalase was shown to prevent small intestinal lesions by inhibiting the generation of superoxide radicals or by scavenging such radicals [31]. On the other hand, the intestinal ulcerogenic response induced by SC-560 plus rofecoxib was also inhibited by PGE₂, which was given 6 h after the administration of these COX inhibitors [16]. N⁶-nitro-L-arginine methyl ester (L-NAME), the nonselective NOS inhibitor, had a biphasic effect on the intestinal ulcerogenic response to indomethacin, depending on the dose schedule; aggravation with its prior administration and protection by its later administration, and these effects were both antagonized by the co-administration of L-arginine [8]. The protective action of cNOS/NO was supported by the finding that NOR-3, a NO donor, prevented the de-
velopment of these intestinal lesions in response to indomethacin [32]. The proloucerogenic effect of L-NAME was also confirmed by the combined administration of rofecoxib together with L-NAME provoking hemorrhagic lesions in the small intestine; however, L-NAME by itself did not cause any damage [33]. The intestinal lesions produced by L-NAME plus rofecoxib were also prevented by a pretreatment with ampicillin and aminoguanidine as well as atropine, similar to the lesions generated by indomethacin [8–10]. These findings suggested that the inhibition of both cNOS and COX-2 provoked intestinal damage, similar to the inhibition of both COX-1 and COX-2.

Cholestyramine, a bile acid sequestrant, dose-dependently prevented the development of small intestinal lesions when given 30 min before the administration of indomethacin, but did not when given 3 h after indomethacin [34]. Similar findings were obtained by bile duct ligation; ligation 30 min before the indomethacin treatment completely prevented the occurrence of intestinal lesions, whereas ligation 3 h after only caused a partial inhibition. Cholestyramine also inhibited bacterial invasion as well as the up-regulation of COX-2 and iNOS expression in the intestinal mucosa after the administration of indomethacin. These findings suggested that bile acids facilitated bacterial invasion in the mucosa and contributed to the upregulated expression of both COX-2 and iNOS; this indicates the necessity of luminal bile acids for the occurrence of NSAID-induced small intestinal damage.

The severity of indomethacin-induced small intestinal damage was reduced by several antiulcer drugs, such as lafutidine, irsogladine, rebamipide, and teprenone [35–40]. The effects of irsogladine were particularly potent and mimicked by rolipram, an inhibitor of phosphodiesterase (PDE) type IV, through a mechanism that is thought to be associated with an increase in the secretion of mucus [35]. Regarding PDE inhibitors, sildenafil, an inhibitor of PDE type V, also potently inhibited indomethacin-induced intestinal lesions, and this effect was attenuated by L-NAME, suggesting a NO/cGMP-dependent action [36]. Lafutidine, a histamine H2-receptor antagonist (H2-RA), also prevented indomethacin- or loxoprofen-induced intestinal lesions, while other H2-RAs did not [37, 39]. This effect of lafutidine was mimicked by capsaicin and totally attenuated by the chemical ablation of capsaicin-sensitive afferent neurons. Although omeprazole, a proton pump inhibitor (PPI), was ineffective against these lesions, another PPI, lansoprazole, reduced the severity of these lesions [37, 40]. Since the protective effect of lansoprazole was attenuated by the prior administration of tin-protoporphyrin IX (SnPP), an inhibitor of hemoxygenase (HO)-1, this action was assumed to be caused by the induction of HO-1 and carbon monoxide. Recent studies reported that antisecretory drugs such as PPIs and H2-RAs exacerbated the intestinal ulcerogenic response to NSAIDs such as indomethacin and naproxen, and this was attributed, at least partly, to dysbiosis of microbiota or intestinal hypermotility [41–43]. These findings suggest the possible risk of the co-administration of NSAIDs and antisecretory drugs; however, this treatment is known to be effective for preventing NSAID-induced gastric and duodenal damage. Watanabe et al. [44] recently examined intestinal damage in 160 arthritic patients who took NSAIDs for more than 3 months and found that both PPIs and H2-RAs were high risk factors for NSAID-induced intestinal damage in humans.

Indomethacin has been reported to cause mucosal lesions as a result of the migration and adhesion of leukocytes to venules in the GI tract, and leukotriene (LT) B4 was shown to be involved in this process [45]. LTC4, LTD4, and LTE4, which are called cysteinyl LTs (cysLTs), have also been reported to cause vascular contraction (ischemia) and increased the permeability of the vasculature feeding the GI tract. However, no clear consensus has yet been reached regarding the role of LTs in the pathogenesis of NSAID-induced intestinal lesions. Indomethacin decreased the content of PGE2, while a 3-fold higher amount of cysLTs in the small intestine was observed in indomethacin-treated rats than in control rats [46]. The intestinal ulcerogenic response to indomethacin was significantly prevented by the pretreatment of pranlukast, a cysLT receptor antagonist. We also showed that these intestinal lesions in cats were significantly inhibited by a pretreatment with a lipoxygenase (LOX) inhibitor (AA-861), in addition to pranlukast [20]. These findings suggest that LTs play a role in the induction of small intestinal lesions by indomethacin.

The intestinal ulcerogenic response to indomethacin was also found to be suppressed by urocortin I, a non-selective corticotropin-releasing factor receptor (CRFR) agonist, and aggravated by astressin, a nonselective CRFR antagonist [47]. Furthermore, the protective effects of urocortin I were reversed by astressin-2B (a CRFR2 antagonist), but not by NBI-27914 (a CRFR1 antagonist). Urocortin I was shown to suppress the hypermotility response to indomethacin, and this effect was also abrogated by astressin-2B, but not by NBI-27914. Endogenous CRF is assumed to contribute to maintain-
ing the mucosal defensive ability of the small intestine against indomethacin through the activation of CRFR2. Recently, a range of NSAID derivatives that release H₂S have been synthesized and evaluated as the GI-sparing NSAIDs, with consistent results in terms of retaining anti-inflammatory activity but reducing GI toxicity [48–50]. The mechanisms by which H₂S can protect against NSAID-enteropathy appear to include changes in the key factors that are central to the pathogenesis of this injury; the composition/secretion of bile, the microbiota and the enterohepatic recirculation of NSAIDs [51].

**Functional Alterations in the Small Intestine after Administration of NSAIDs**

**Intestinal Motility**

Intestinal hypermotility has been implicated as one of the pathogenic factors in NSAID-induced small intestinal lesions [10, 19, 20]. NSAIDs such as indomethacin markedly enhance intestinal motility, both the amplitude and frequency of contractions at the ulcerogenic dose (fig. 2b). In all cases, the contractile activity of the small intestine started to increase within 30–50 min of the administration, and the hypermotility response persisted for over 3 h. SC-560 also caused intestinal hypermotility, whereas rofecoxib did not. The enhanced intestinal motility caused by indomethacin was inhibited by the subsequent administration of PGE₂ and atropine. Neither aminoguanidine nor ampicillin had any effect on the enhanced intestinal motility caused by indomethacin [19]. As discussed earlier, intestinal damage occurs due to the inhibition of both cNOS and COX-2 as well as COX-1 and COX-2 [16, 33]. The inhibition of NO production by L-NAME caused an increase in intestinal motility, resulting in an enhancement in bacterial invasion, which are events similar to those observed under the conditions of a PG deficiency caused by SC-560 through the inhibition of COX-1. However, the inhibition of cNOS or COX-1 upregulated the expression of COX-2 through intestinal hypermotility, and this may have counteracted the deleterious events caused by an endogenous NO or PG deficiency [16, 33]. The intestinal hypermotility response to indomethacin was also confirmed in cats, especially in the ileum, and this response was inhibited by AA-861, pranlukast, a PGE₁ derivative (misoprostol), and atropine [20], which suggested the involvement of LTs, in addition to a PG deficiency and cholinergic intervention, in the mechanism underlying hypermotility. Intestinal hypermotility may break down the mucus layer due to mucosal rubbing and accelerates the invasion of intestinal bacteria into the mucosa.

**Enterobacterial Invasion**

The number of enterobacteria was previously reported to be markedly increased in the intestinal mucosa under both aerobic and anaerobic conditions following the administration of NSAIDs [8, 9, 18, 19, 52]. SC-560, with or without the co-administration of rofecoxib, also significantly increased the mucosal invasion of enterobacteria, while rofecoxib alone had no effect [18]. Bacterial invasion in the intestinal mucosa following indomethacin was significantly prevented by the prior administration of the antibiotic ampicillin, with the numbers of both aerobic and anaerobic bacteria decreasing to below the control levels observed in normal mucosa. Both PGE₂ and atropine, but not aminoguanidine, suppressed this increase in bacterial invasion in the mucosa following the administration of indomethacin [19, 53]. Wallace et al. [41] demonstrated that a 9-days treatment with omeprazole significantly reduced Actinobacteria and Bifidobacteria spp. in the rat jejunum, and suggested that PPIs exacerbated NSAID-induced intestinal damage through dysbiosis. Although the mechanism by which enterobacteria invade the mucosa currently remains unknown, previous studies suggested that a decrease in the secretion of mucus may have contributed to this process following the indomethacin treatment [10, 35, 39]. Since mucus plays a crucial role in innate host defenses against intestinal pathogens and irritants, a decrease in mucus secretion may weaken the intestinal barrier, resulting in bacterial invasion. A previous study demonstrated that loxoprofen decreased the expression of Muc2 mRNA in the small intestine [39]. Muc2, an important mucin, plays a major role in the dimerization of secretory mucin, an essential step in the formation of GI mucus gels [54]. Mucosal protective drugs were found to upregulate the expression of Muc2 and mucus secretion, thereby increasing the thickness of the mucus gel and hampering bacterial invasion following the administration of NSAIDs [35, 39, 55]. The secretion of intestinal fluid was also shown to prevent bacterial invasion by washing out the invading bacteria [10, 35, 56, 57].

**iNOS and TNFα**

An RT-PCR analysis revealed that neither iNOS nor TNFα mRNAs was detected in the normal intestinal mucosa, but they were potently expressed in the mucosa as early as 3 h after the administration of indomethacin [56,
The upregulated expression of iNOS mRNA was similarly observed in the intestinal mucosa of animals given SC-560, but not rofecoxib [16]. The expression of iNOS mRNA and the increase observed in iNOS activity following indomethacin were inhibited by the prior administration of PGE2 and ampicillin as well as atropine (fig. 2c); however, none of these agents had any effect on the constitutive expression of NOS in the intestinal mucosa [18, 32]. The content of NO in the intestinal mucosa 24 h after the treatment with indomethacin was more than 3-fold higher than basal values [8, 9, 53], and this response was inhibited by either L-NAME or aminoguanidine. Although a small amount of NO was produced in normal rats not treated with indomethacin, this production was reduced by L-NAME, but not by aminoguanidine. NO is known to interact with superoxide radicals to produce a cytotoxic peroxynitrite, which has a deleterious influence on GI mucosal integrity [58]. Rachmilewitz et al. [30] induced the tissue inflammation by applying a peroxynitrite-generating system to the rat colonic mucosa, while Miller et al. [59] demonstrated the expression of iNOS and formation of peroxynitrite in guinea pig ileitis. We previously reported that the severity of indomethacin-induced small intestinal lesions was reduced by a pretreatment with allopurinol, a xanthine oxidase inhibitor, as well as hydroxyurea, a neutrophil-reducing agent [31]. Thus, the detrimental role of NO in NSAID-induced small intestinal lesions may be explained by the cytotoxic effects of peroxynitrite produced from NO in the presence of superoxide radicals.

On the other hand, Bertrand et al. [60] demonstrated that NSAIDs induced the local production of TNFa in the small intestine and this event occurred prior to elevations in the production of NO and MPO activity as well as lesion formation. However, Reuter and Wallace [61] reported that TNFa did not play a critical role in NSAID-induced small intestinal injuries, because the inhibited release of TNFa by thalidomide or immunoneutralization with a polyclonal antibody against TNFa failed to afford any protection against indomethacin-induced small intestinal lesions. Thus, controversy remains over the involvement of TNFa in the pathogenesis of NSAID-induced enteropathy. We reported that ampicillin, cinacalcet, or lubiprostone prevented the upregulation of iNOS and TNFa as well as the invasion of enterobacteria in the intestinal mucosa following the administration of indomethacin, which suggested a close relationship between the mucosal invasion of enterobacteria and the upregulation of these cytokines [19, 33, 56, 57].

Up-Regulation of COX-2 Expression

Although the gene expression of COX-2 was shown to be negligible in the normal rat intestine, the expression of COX-2 mRNA was upregulated in the rat intestine 6 h after the administration of conventional NSAIDs at ulcerogenic doses (fig. 3a) [16]. The upregulation of COX-2 was similarly observed in the rat small intestine as early as 3 h after the administration of SC-560, but not rofecoxib (fig. 3b). COX-1 mRNA was observed in the intestinal mucosa of rats, irrespective of whether the animal was treated with conventional NSAIDs, SC-560, or rofecoxib. On the other hand, indomethacin decreased the PGE2 content in the intestinal mucosa within 3 h, and these values remained reduced for up to 24 h later. SC-560 decreased the mucosal PGE2 content as effectively as indomethacin when determined 3 h after its administration, while the reduced PGE2 level gradually recovered from 6 h later and was almost totally restored to basal values 12 h later (fig. 3c). This recovery in the content of PGE2 was significantly prevented by rofecoxib, given together with SC-560. Rofecoxib alone had no effect on the mucosal PGE2 content in the rat intestine at any time point. The upregulation of COX-2 expression by SC-560 was inhibited by the prior administration of PGE2, ampicillin, or atropine [62]. Neither of these agents had any effect on the expression of COX-1 in the intestinal mucosa. Similar findings were obtained when the expression of COX-2 was induced by indomethacin, instead of SC-560. The mucosal PGE2 content observed 12 h after the administration of SC-560 was significantly decreased by the co-administration of rofecoxib. Similarly, the recovery of PGE2 after SC-560 was also significantly prevented when animals were pretreated with either ampicillin or atropine at doses that inhibited the upregulation of COX-2 expression.

Toll-Like Receptor 4

Toll-like receptor (TLR) 4 recognizes lipopolysaccharide (LPS), the endotoxin of Gram-negative bacteria, and activates an inflammatory cascade via the accessory protein MyD88. Ampicillin and aztreonam decreased the number of Gram-negative bacteria in the contents of the rat small intestine and inhibited indomethacin-induced intestinal damage [9, 63]. However, vancomycin, which exhibited no activity against Gram-negative bacteria, had no preventive effect against these lesions. LPS, given 1 h after indomethacin, aggravated the intestinal ulcerogenic response, whereas a pretreatment with LPS inhibited this response with a reduction in the expression of TLR4 and cytokines. Both lesion formation and cytokine expression
were markedly inhibited in TLR4-mutant mice as well as MyD88 (−/−) mice. Based on these findings, Watanabe et al. [63] suggested that Gram-negative bacteria played a major pathogenic role in the intestinal ulcerogenic response to indomethacin through a TLR4/MyD88-dependent pathway. We previously reported that the intestinal ulcerogenic response to indomethacin was aggravated in arthritic rats, and this phenomenon may be attributable to the upregulation of iNOS/NO through the increased expression of TLR4 in the small intestine of arthritic rats [64].

**Collagen I and Autophagy**

Recent studies showed novel mechanisms underlying indomethacin-induced intestinal cell damage in vitro. Edogawa et al. [65] suggested the role of the collagen synthesis in intestinal mucosa in the mechanism of NSAID-induced small intestinal lesions. They showed that indomethacin reduced the expression of collagen I and the proteins related to collagen I synthesis and maturation and also demonstrated the protective effect of collagen on intestinal mucosal cells by the use of a collagen-synthesis inhibitor or siRNA knockdown of endogenous collagen I. Narabayashi et al. [66] reported that indomethacin induced accumulation of cytoplasmic lipid droplets in cultured enterocytes, in association with time-dependent autophagic responses. A predominant cytoprotective lipophagy was initially activated in indomethacin-treated enterocytes, and after prolonged exposure to indomethacin there was a decrease of lysosome-associated membrane protein 2 expression in the enterocytes in addition to an increase of lipoapoptosis. It remains, however, unknown whether these results observed in enterocytes in vitro may account for the onset of intestinal damage in vivo, because NSAID-induced enteropathy in vivo was all but completely inhibited by ampicillin [19, 53] and NSAIDs did not damage the small intestine in germ-free animals or fasting animals [1, 53].

**Myeloperoxidase (MPO) Activity**

MPO activity, representing neutrophil infiltration in the mucosa, was shown to be markedly elevated from approximately 6 h after the administration of indomethacin, SC-560 or rofecoxib in rats. Animals were administered indomethacin (10 mg/kg), SC-560 (10 mg/kg), rofecoxib (10 mg/kg) p.o. and then killed 3, 6 and 12 h later. Data are presented as the mean ± SE from 6 rats. Significant difference at p < 0.05: * from normal; # from SC-560 at 12 h (from ref. [16] after modification).

![Fig. 3. Gene expression of COX-1, COX-2, and GAPDH in the rat intestinal mucosa after the administration of various NSAIDs (a) and selective COX inhibitors (b). Animals were administered indomethacin (IM: 10 mg/kg), diclofenac (DIC: 40 mg/kg), flurbiprofen (Flu: 20 mg/kg), naproxen (NAP: 40 mg/kg), SC-560 (SC: 10 mg/kg), rofecoxib (Rof: 10 mg/kg) p.o. and then killed 6 h later. M: marker, V: vehicle. c Time-course of changes in the mucosal PGE2 content after the administration of indomethacin, SC-560 or rofecoxib in rats. Animals were administered indomethacin (10 mg/kg), SC-560 (10 mg/kg), rofecoxib (10 mg/kg) p.o. and then killed 3, 6 and 12 h later. Data are presented as the mean ± SE from 6 rats. Significant difference at p < 0.05: * from normal; # from SC-560 at 12 h (from ref. [16] after modification).](image-url)
Neither SC-560 nor rofecoxib alone increased MPO activity in the intestinal mucosa; however, the combined administration of these two agents led to significantly higher MPO activity than that observed in normal rats [18]. It is assumed that COX-2/PGE₂ helps to maintain the integrity of the intestinal mucosa by inhibiting the migration of neutrophils on inhibition of COX-1. The increase in MPO activity in response to indomethacin was significantly suppressed by the treatment of animals with PGE₂, ampicillin, and aminoguanidine as well as atropine [19]. Neutrophils are recruited to a site of injury by chemotaxins and participate in amplifying the inflammatory response by releasing several chemotaxins and producing further tissue injury through the release of reactive oxygen metabolites [58]. These blood cells are also a source of iNOS, and peroxynitrites formed by the interaction between NO and oxygen radicals may be detrimental in inflammatory lesion models [30, 59].

Relevance of the Functional Changes to Human Study
Some of the functional changes induced by NSAIDs were observed not only in animals but also in humans. The roles of mucus secretion, microbiota, and inflammatory cytokines have been confirmed in patients [17, 52]. However, the enhanced intestinal motility, the most important pathogenic element in NSAID-induced enteropathy, has not been recognized in patients, because no study was conducted to examine whether NSAIDs cause hypermotility response in human. Notwithstanding, as we wrote in the text, atropine, an antimuscarinic drug, inhibited the hypermotility response and prevented small intestinal lesions following the administration of NSAIDs. Any pathogenic hypothesis cannot explain how atropine totally prevented the development of NSAID-induced enteropathy. So, we believe that the functional alterations induced in rodent small intestines by NSAIDs could be observed in human small intestines.

Roles of EP Receptor Subtypes in Protective Effects of PGE₂
Although several factors have been postulated as pathogenic elements of NSAID-induced enteropathy, a deficiency of PGs has clearly been shown to play a critical role in the pathogenesis of these lesions [7, 16]. All these events caused by NSAIDs are effectively prevented by supplementation with exogenous PGE₂ or its derivative [10, 18, 19]. We used prostanoids, subtype-specific EP receptor agonists and antagonists, as a tool, and characterized the EP receptor subtypes related to the protective effects of PGE₂ against these lesions [10].

The development of indomethacin-induced small intestinal lesions was dose-dependently prevented by the prior administration of PGE₂ [10]. Other prostanoids such as ONO-NT-012 (an EP3 agonist) and ONO-AE1–329 (an EP4 agonist) provided dose-dependent protection against indomethacin-induced intestinal damage, whereas neither 17-phenyl PGE₂ (an EP1 agonist) nor butaprost (an EP2 agonist) had any effect on these lesions. Lubiprostone, a bicyclic fatty acid derived from PGE₁ [67], also prevented these lesions in the small intestine, and this effect was significantly abrogated by the co-administration of AE3–208, an EP4 antagonist, suggesting the involvement of EP4 receptors in these protective effects [57]. These findings support the importance of EP4 receptors in the protective effects of PGE₂ against NSAID-induced intestinal damage.

The increase in bacterial translocation and iNOS expression following the administration of indomethacin was markedly prevented both by a pretreatment with PGE₂, and these effects were reproduced by NO-NT-012, ONO-AE1–329 and lubiprostone, but not by 17-phenyl PGE₂ or butaprost [10, 57]. These prostanoids increased the amount of mucus secreted in the small intestine, suggesting the involvement of EP3/EP4 receptors in these protective effects [57]. These findings support the importance of EP4 receptors in the protective effects of PGE₂ against NSAID-induced intestinal damage.
Can-C
Can-B
Dry-C
Dry-A
Dry-B
fed canned food supplemented with cellulose (3 and 6%), low amount of DF (<0.4%). However, when animals were feeding various canned foods (Can-A, B and C) or dry foods (Dry-A, B and C) containing various concentrations of dietary fiber (0–7.2%). Intestinal lesions were examined 24 h after the final dosing of indomethacin. Data are presented as the mean ± SE from 5 or 6 rats (from ref. [21] after modification).

**Role of Foods in the Intestinal Ulcerogenic Response to Indomethacin**

The location of GI lesions caused by NSAIDs in the rat is known to depend upon food intake; that is, lesions were mainly detected in the stomach of fasted animals, with almost no lesions being observed in the small intestine. However, lesions were observed predominantly in the small intestine of conventionally fed rats given chow pellets containing dietary fiber (DF, 4.2%). When animals were fed a liquid diet containing no DF, indomethacin did not cause any lesions in the small intestine; however, indomethacin clearly caused lesions in a concentration-dependent manner when cellulose (1–10%) was added to the liquid diet [70]. These findings suggested that DF such as cellulose is harmful for small intestinal lesions induced by NSAIDs.

NSAIDs are often administered to dogs and cats to treat various diseases, and sometimes induce GI damage at clinical doses, similar to humans. The importance of DFs in the intestinal ulcerogenic response to NSAIDs has also been confirmed in cats [20, 21]. The severity of small intestinal lesions induced by indomethacin depended on the concentration of DFs (0–7.2%) in canned and dry foods (fig. 4). Furthermore, indomethacin did not cause intestinal lesions in cats given canned food containing a low amount of DF (<0.4%). However, when animals were fed canned food supplemented with cellulose (3 and 6%), indomethacin dose-dependently produced intestinal lesions [21]. Recently, we examined the role of food in aspirin-induced small intestinal lesions in cats, and almost the same findings as those with indomethacin were obtained [71]. Indomethacin also increased motility in the lower intestine, in which lesions are often observed. These findings suggested that mucosal injury could be caused by rubbing of the mucosa with the undigested solid components of food such as cellulose under intestinal hypermotility especially when the secretion of mucus is decreased by NSAIDs [20, 21].

On the other hand, the addition of 3% soluble DF (SDFs: pectin, guar gum, or polydextrose) to regular dry food significantly decreased indomethacin-induced intestinal lesion formation [21]. These findings indicated that SDFs (such as pectin), in contrast to insoluble DFs (such as cellulose), protected the small intestine against NSAID-induced mucosal damage. Similar to SDFs, 3% mucin extracted from the pig stomach also decreased indomethacin-induced intestinal lesions. Both SDFs and mucin have similar polysaccharide structures and form gels when dissolved in water, and their viscosities increase in a concentration-dependent manner. A strong correlation was also observed between viscosity and the protective action of SDFs, that is, guar gum > pectin > mucin ≥ polydextrose. These findings suggested that SDFs protected the intestinal mucosa by compensating for the barrier function of mucin, which was decreased by NSAIDs.

**Intestinal Ulcerogenic Action of Aspirin**

Conventional NSAIDs damage the small intestine with a concomitant decrease in mucosal PGE₂ production, irrespective of the route of administration [16]. However, since aspirin is not ulcerogenic in the small intestine, even though it reduces mucosal PGE₂ production as effectively as other NSAIDs [22], it is likely that the depletion of endogenous PGs by itself is not sufficient for the formation of intestinal lesions and other factors are required for the onset of intestinal damage. Robert et al. [72, 73] reported that aspirin showed an anti-ulcer effect against various experimental ulcer models including gastrointestinal lesions. We confirmed that aspirin did not damage the small intestine but dose-dependently inhibited indomethacin-induced intestinal injury [22] (fig. 5a). This protective effect of aspirin was mimicked by salicylic acid, with its action being more potent than that of aspirin. Since high levels of salicylic acid, the major metabolite of aspirin, were detected in blood following the ad-
administration of aspirin [22, 23], it is possible that its protective effects may be mediated by salicylic acid. Both aspirin and salicylic acid significantly prevented bacterial invasion in the mucosa, which plays a critical role in the development of intestinal lesions following the administration of indomethacin (fig. 5b). The reason why aspirin does not induce intestinal damage may be explained, at least partly, by the protective effects of salicylic acid. Aspirin and salicylic acid did not increase intestinal motility, but suppressed the enhanced motility response following an indomethacin treatment, which again suggested a relationship between the inhibition of intestinal hypermotility and prevention of intestinal damage (fig. 5c). Since no study to test the protective effect of parenteral aspirin in humans is currently available, further examination should be needed to prove the clinical effectiveness of aspirin against NSAID-induced enteropathy.

**Summary and Future Prospects**

Conventional NSAIDs, such as indomethacin, have been shown to produce hemorrhagic lesions in the rat small intestine. In addition to a PG deficiency due to the inhibition of COX-1, several factors have been suggested in the pathogenic mechanism of these lesions, such as in-
Increased intestinal motility, decreased mucus secretion, enterobacterial invasion, neutrophil migration, and the upregulation of iNOS expression/NO production, which eventually cause intestinal damage [8, 9, 16, 19] (fig. 6). However, the inhibition of COX-1 has been shown to upregulate the expression of COX-2, and PGs produced by COX-2 may suppress the detrimental processes associated with the inhibition of COX-1, including increases in MPO and iNOS activity [16]. Furthermore, since ampicillin prevented the expression of COX-2 after treatment with indomethacin, even though it had no effect on intestinal hypermotility, the effects of the expression of COX-2 on the inhibition of COX-1 are assumed to be closely associated with intestinal hypermotility and subsequent bacterial invasion. These sequential events related to COX-1 and/or COX-2 inhibition may explain why intestinal damage occurs only when both COX-1 and COX-2 are inhibited. It is assumed that COX-2 as well as COX-1 play a role in maintaining the mucosal integrity of the small intestine and that the inhibition of both COX-1 and COX-2 is required for the intestinal ulcerogenic properties of NSAIDs to occur. In addition, the inhibition of both cNOS and COX-2 was previously reported to provoke intestinal damage, similar to the inhibition of both COX-1 and COX-2 [33]. The inhibition of cNOS, similar to COX-1, upregulates the expression of COX-2, a process associated with intestinal hypermotility and bacterial invasion. No satisfactory means for the treatment of NSAID-induced enteropathy are currently available for patients. This situation has been markedly intensified by...
the recent findings that antisecretory drugs, such as PPIs and H$_2$–RAs, are ineffective against NSAID-induced small intestinal damage [37, 38] and even worsen the severity of these lesions [41–43]. Furthermore, it was also found that soluble dietary fibers protect the small intestine against NSAID-induced damage, probably by compensating for a decreased in barrier function caused by NSAIDs. Thus, the identification of effective therapies for the treatment of NSAID-induced small intestinal lesions remains an urgent priority.

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Disclosure Statement

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