Nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin decrease mucosal PGE\textsubscript{2} production by inhibiting cyclooxygenase (COX) activity and produce damage in the small intestine. The development of intestinal lesions as induced by indomethacin was accompanied by increases in intestinal motility, enterobacterial invasion, and myeloperoxidase (MPO) as well as inducible nitric oxide synthase (iNOS) activity, together with the up-regulation of COX-2 and iNOS mRNA expression. Neither the selective COX-1 inhibitor, SC-560, nor the selective COX-2 inhibitor, rofecoxib, alone caused intestinal damage, but their combined administration produced lesions. SC-560, but not rofecoxib, caused intestinal hypermotility, bacterial invasion and the expression of COX-2 as well as iNOS mRNAs, yet the iNOS and MPO activity was increased only when rofecoxib was administered together with SC-560. Although SC-560 inhibited the PG production, the level of PGE\textsubscript{2} was recovered, in a rofecoxib-dependent manner. The intestinal hypermotility response to indomethacin was prevented by both 16,16-dimethyl PGE\textsubscript{2}, and atropine but not ampicillin, yet all these agents inhibited not only the bacterial invasion but also the expression of COX-2 as well as the iNOS activity in the intestinal mucosa following indomethacin treatment, resulting in preventing the intestinal lesions. These results suggest that inhibition of COX-1, despite causing intestinal hypermotility, bacterial invasion and iNOS expression, up-regulates the expression of COX-2, and the PGE\textsubscript{2} derived from COX-2 counteracts deleterious events caused by COX-1 inhibition and maintains the mucosal integrity. These sequences of events explain why intestinal damage occurs when both COX-1 and COX-2 are inhibited.

**Key words:** NSAID, Intestinal damage, pathogenic mechanism, COX-1 inhibition, COX-2 inhibition
INTRODUCTION

Non-steroidal antiinflammatory drugs (NSAIDs) such as indomethacin, after short-term and long-term administration, cause intestinal ulceration in human and laboratory animals (1-3). Although several factors are involved in the pathogenesis of these lesions, including a deficiency of prostaglandins (PGs), bile acid, bacterial flora and nitric oxide (NO) (4-8), a deficiency of endogenous PGs is of prime importance in the background for the intestinal ulcerogenic response to NSAIDs. This contention is supported by the fact that NSAID-induced intestinal damage is prevented by supplementation with exogenous PGE$_2$ (9, 10).

PG deficiency caused by NSAIDs is due to cyclooxygenase (COX) inhibition. The COX exists in two isoforms, the constitutively expressed COX-1 and the inducible COX-2. The former is found normally in various tissues, including the small intestine (11), while the latter does not appear to be expressed, or at least at very low levels, in most tissues and is rapidly up-regulated in response to growth factors and cytokines (12-14). This tissue specificity leads to the idea that COX-1 is critical for housekeeping action in the gastrointestinal mucosa (15, 16), whereas COX-2 is responsible for inflammation (17, 18). Studies using selective COX-2 inhibitors showed that the ulcerogenic property of NSAIDs in the gastrointestinal tract is brought about by inhibition of COX-1 but not COX-2 (15, 19). However, recent studies showed that inhibition of both COX-1 and COX-2 is required for NSAID-induced ulceration.

![Fig. 1. Intestinal ulcerogenic response of various NSAIDs and the effect of these agents on the mucosal PGE$_2$ content in the rat small intestine. The animals were administered indomethacin (IND: 10 mg/kg), diclofenac (DIC: 40 mg/kg), flurbiprofen (FLU: 20 mg/kg) or naproxen (NAP: 40 mg/kg) p.o., and killed 3 or 24 hr later for determination of PGE$_2$ or lesions, respectively. Data are presented as the mean±SE from 5-6 rats. *Significant difference from vehicle, at p<0.05. (Unpublished data).]
gastrointestinal injury, suggesting a role of COX-2 as well as COX-1 in maintaining the mucosal integrity of these tissues (20-23).

In this article, we reviewed our recent publications on the pathogenesis of NSAID-induced intestinal damage and discuss the relation of COX-1 or COX-2 inhibition with various pathogenic elements of these lesions, including PG

![Intestinal Lesions (mm²)](image)

**Fig. 2.** Effect of SC-560 and rofecoxib on intestinal mucosa and PGE₂ content in the rat. The animals were administered SC-560 (10 mg/kg) or rofecoxib (10 mg/kg) p.o., either alone or in combination, and killed 3 or 24 hr later for determination of PGE₂ or lesions, respectively. Data are presented as the mean±SE from 4–6 rats. *Significant difference from vehicle, at p<0.05. (Unpublished data).

![Damage Score (mm²)](image)

**Fig. 3.** Effects of various agents on indomethacin-induced intestinal lesions in rats. Animals were given indomethacin p.o. at a dose of 10 mg/kg and killed 24 hr later. DMPGE₂ (30 µg/kg), atropine (3 mg/kg) and aminoguanidine (20 mg/kg) were given s.c. twice 30 min before and 9 hr after indomethacin while ampicillin (800 mg/kg) was given p.o. twice 24 hr and 30 min before indomethacin. Data are presented as the mean±SE from 5–6 rats. *Significant difference from vehicle, at P<0.05. (Unpublished data).
deficiency, intestinal motility, neutrophil infiltration and NO production (8-10, 21-24).

INTESTINAL ULCEROGENIC PROPERTIES

Nonselective COX inhibitors

Conventional NSAIDs, such as indomethacin, diclofenac, flurbiprofen and naproxen, produced hemorrhagic damage in the small intestinal mucosa within 24 hr, mainly in the jejunum and ileum (Fig. 1). The apparent size and morphology of intestinal lesions were similar, irrespective of which NSAID was used to induce the damage or which route of administration was used for dosing (p.o. or s.c.). All nonselective COX inhibitors at the ulcerogenic doses caused a marked decrease in the mucosal PGE$_2$ content of the small intestine.

Selective COX inhibitors

Neither the selective COX-1 inhibitor SC-560 nor the selective COX-2 inhibitor rofecoxib caused any damage the small intestine within 24 hr (Fig. 2). However, these drugs when given together 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 was increased depending upon the dose of rofecoxib. The same was observed when rofecoxib
was administered together with increasing doses of SC-560. The intestinal ulcerogenic response induced by SC-560 plus rofecoxib was significantly inhibited by 16,16-dimethyl PGE\(_2\) (dmPGE\(_2\)) given 6 hr after administration of these COX inhibitors (22). Rofecoxib had no effect on the mucosal PGE\(_2\) content of the small intestine, while SC-560 at 10 mg/kg caused a significant decrease in PGE\(_2\) content, the effect being equivalent to that induced by indomethacin at 10 mg/kg.

**EFFECT OF VARIOUS DRUGS ON THE ULCEROGENIC RESPONSE**

The development of indomethacin-induced intestinal damage was significantly prevented by supplementation of dmPGE\(_2\) (Fig. 3). Ampicillin, the antibiotics, also significantly reduced the severity of intestinal damage in response to indomethacin. Furthermore, these lesions were prevented by the selective iNOS inhibitor aminoguanidine or the anticholinergic drug atropine. However, the effect of \(\text{NO}^\circ\)-nitro-L-arginine methyl ester (L-NAME), the nonselective NOS inhibitor, showed a dual effect on the intestinal ulcerogenic response to indomethacin depending on the time of dosing; the aggravation by the prior administration and the protection by the later administration, and the effects were both antagonized by co-administration of L-arginine (Fig. 4). Furthermore, L-NAME, given twice 30 min before and 6 hr after indomethacin, did not significantly affect the severity of lesions, yet even in this case, when L-arginine was given together with the first injection of L-NAME, the severity of damage

![Fig. 5. Gene expression of COX-1, COX-2 and G3PDH in the rat intestinal mucosa following administration of various NSAIDs (A) and selective COX inhibitors (B). (A) The animals were administered indomethacin (IM: 10 mg/kg), diclofenac (DIC: 40 mg/kg), flurbiprofen (Flu: 20 mg/kg) and naproxen (NAP: 40 mg/kg) p.o., and killed 6 hr later. (B) The animals were administered indomethacin (IM: 10 mg/kg), SC-560 (SC: 10 mg/kg) or rofecoxib (Rof: 10 mg/kg) p.o., and killed 6 hr later. M: marker, V: vehicle. (Unpublished data).]
was markedly suppressed, just like the case observed by the later administration of L-NAME.

**COX-2 EXPRESSION AND THE RELATED PGE2 PRODUCTION**

Although the gene expression of COX-2 was negligible in the normal rat intestine, the expression of COX-2 mRNA was up-regulated in the rat intestine when examined at 6 hr after administration of conventional NSAIDs at the ulcerogenic doses (Fig. 5A). The up-regulation of COX-2 was similarly observed in the rat small intestine as early as 3 hr after administration of SC-560 but not rofecoxib (Fig. 5B). Both G3PDH and COX-1 mRNAs were observed in the intestinal mucosa of rats, irrespective of whether or not the animal was treated with conventional NSAIDs, SC-560 or rofecoxib. On the other hand, indomethacin (10 mg/kg) markedly decreased the PGE$_2$ content in the intestinal mucosa within 3 hr, and the values remained lowered up to 24 hr later (Fig. 6). SC-560 (10 mg/kg) decreased the mucosal PGE$_2$ content as effectively as indomethacin when determined 3 hr after the administration, yet the reduced PGE2 level was gradually recovered from 6 hr later and almost totally restored to the basal values 12 hr later. This recovery of PGE$_2$ content was significantly prevented by rofecoxib given together with SC-560. Rofecoxib alone had no effect on the mucosal PGE$_2$ content in the rat intestine at any time points.
ALTERATION OF INTESTINAL FUNCTIONS

Intestinal motility

Intestinal hypermotility has been implicated as one of the pathogenic factors in NSAID-induced small intestinal lesions (10, 24). Under urethane anesthesia, no clear contraction was observed in the small intestine of normal rats, resulting in a fluctuation at baseline levels. NSAIDs such as indomethacin caused a marked enhancement of intestinal motility at the ulcerogenic doses, with regard to both the amplitude and frequency of contraction (Fig. 7). In all cases, the contractile activity of the small intestine started to increase within 30-50 min after the administration, and the hypermotility response persisted for over 3 hr. SC-560 also caused intestinal hypermotility, while rofecoxib had no influence on intestinal motility (Fig. 8). The enhanced intestinal motility caused by indomethacin was markedly inhibited by subsequent administration of dmPGE₂ and atropine. Neither ampicillin nor aminoguanidine had any effect on the enhanced intestinal motility seen after administration of indomethacin (Fig. 9 and Table 1).
Fig. 8. Effects of various COX inhibitors on intestinal motility in rats. Indomethacin (10 mg/kg), SC-560 (10 mg/kg) or rofecoxib (10 mg/kg) was given i.d. after basal intestinal motility had well stabilized. Data were expressed as a motility index (arbitrary units) and are presented as the mean±SE from 4~6 rats. *Significant difference from vehicle, at p<0.05. (Unpublished data).

Fig. 9. Representative recordings showing the effects of various drugs on intestinal hypermotility induced by indomethacin in rats. Indomethacin (10 mg/kg) was given s.c. after basal motor activity had stabilized. Either dmPGE\(_2\) (30 µg/kg), atropine (3 mg/kg) or aminoguanidine (20 mg/kg) was given s.c. 2 hr after indomethacin, while ampicillin (800 mg/kg) was given i.d. 24 hr before indomethacin treatment. (Unpublished data).
Enterobacterial invasion

The number of enterobacteria in both aerobic and anaerobic conditions were markedly increased in the intestinal mucosa following administration of indomethacin. Likewise, SC-560, with or without coadministration of rofecoxib, 

Table 1. Effect of Aminoguanidine on Nitrite/Nitrate Content in The Small Intestinal Mucosa Following Administration of Indomethacin in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Numbers of Rats</th>
<th>Nitrite/Nitrate Content (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline</td>
<td>5</td>
<td>166.5 ± 7.5</td>
</tr>
<tr>
<td>L-MAME</td>
<td>6</td>
<td>97.3 ± 4.5*</td>
</tr>
<tr>
<td>Aminoguanidine</td>
<td>5</td>
<td>168.4 ± 9.1</td>
</tr>
<tr>
<td>Normal Indomethacin</td>
<td>5</td>
<td>168.5 ± 22.5</td>
</tr>
<tr>
<td>+Vehicle</td>
<td>5</td>
<td>505.6 ± 43.5*</td>
</tr>
<tr>
<td>+L-NAME</td>
<td>5</td>
<td>111.5 ± 19.3#</td>
</tr>
<tr>
<td>+Aminoguanidine</td>
<td>5</td>
<td>199.3 ± 42.8#</td>
</tr>
</tbody>
</table>

All values are presented as the mean±SE from 5–6 rats per group. In normal rats, the animals were given L-NAME (20 mg/kg) or aminoguanidine (20 mg/kg) s.c., and killed 1 hr later. In some cases, indomethacin (10 mg/kg) was given s.c. 6 hr before administration of L-NAME or Aminoguanidine, and the animals were killed 24 hr after indomethacin treatment. Significant difference at P<0.05: *from normal (vehicle); # from vehicle. (Unpublished data).

Fig. 10. Gene expression of iNOS and G3PDH in the rat intestinal mucosa following administration of indomethacin, SC-560 or rofecoxib (A) and the effect of various drugs on the iNOS expression induced by indomethacin (B). In Fig. A, the animals were administered indomethacin (IM: 10 mg/kg), SC-560 (SC: 10 mg/kg) or rofecoxib (Rof: 10 mg/kg) p.o., and killed 6 hr later. In Fig. B, dmPGE$_2$ (10 µg/kg) or atropine (3 mg/kg) was given s.c. 30 min before administration of indomethacin (10 mg/kg), while ampicillin (800 mg/kg) was given p.o. twice 24 hr and 30 min before indomethacin. M: marker, V: vehicle. (Unpublished data).
significantly increased the bacterial count in the mucosa, although rofecoxib alone had no effect (Table 1). The bacterial invasion in the intestinal mucosa following indomethacin was completely blocked by prior administration of the antibiotic ampicillin, the numbers of both aerobic and anaerobic bacteria decreasing even below control levels seen in the normal mucosa. Both dmPGE$_2$ and atropine but not aminoguanidine also suppressed the increase in bacterial invasion in the mucosa in response to indomethacin.

**Induction of iNOS and its activity**

RT-PCR analysis revealed that iNOS mRNA was not detected in the normal intestinal mucosa but potently expressed in the mucosa as early as 3 hr after administration of indomethacin (Fig. 10A). The up-regulation of iNOS mRNA expression in the intestinal mucosa was similarly observed in the animals given

| Intestinal Functional Alterations Induced by Indomethacin and Effects of Various Drugs |
|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| **Indomethacin**                           | **Intestinal Hypermotility**                | **Entero bacterial Invasion**                | **iNOS Activity**                          | **MPO Activity**                           | **Intestinal Mucosa**                      |
| Indomethacin                               | increase                                   | increase                                   | increase                                   | increase                                   | Lesions                                    |
| (+) dmPGE2                                 | ↓                                          | ↓                                          | ↓                                          | ↓                                          | ↓                                         |
| (+) Atropine                                | ↓                                          | ↓                                          | ↓                                          | ↓                                          | ↓                                         |
| (+) Aminoguanidine                         | no effect                                  | no effect                                  | ↓                                          | ↓                                          | ↓                                         |
| (+) Ampicillin                             | no effect                                  | ↓                                          | ↓                                          | ↓                                          | ↓                                         |

↓ : Inhibition

Indomethacin (10 mg/kg) produces an increase in intestinal motility, enterobacterial invasion, MPO activity and NO production due to iNOS expression, and results in severe hemorrhagic lesions in the small intestine. DMPGE2 (30 µg/kg) as well as atropine (3 mg/kg) exhibit an inhibitory influence on all these functional changes and prevent the intestinal ulcerogenic response to indomethacin. Ampicillin (800 mg/kg), despite having no effect on the hypermotility response, blocks the bacterial invasion and later events including the development of intestinal lesions. Likewise, aminoguanidine (20 mg/kg) does not have any effect on the intestinal hypermotility response, but prevents indomethacin-induced intestinal lesions by suppressing iNOS/NO production. (Unpublished data).
SC-560 but not rofecoxib. In addition, the expression of iNOS mRNA following indomethacin was apparently prevented by prior administration of dmPGE$_2$ and ampicillin as well as atropine (Fig. 10B). As expected, indomethacin markedly increased iNOS activity in the intestinal mucosa when determined 24 hr later. The iNOS activity was not affected by either SC-560 or rofecoxib, but significantly increased in the animals treated with SC-560 plus rofecoxib. The increase in iNOS activity following indomethacin was significantly reduced by either dmPGE$_2$, ampicillin or atropine, although neither of these agents had any effect on constitutive NOS (cNOS) activity in the intestinal mucosa. On the other hand, the NO content in the intestinal mucosa increased more than 3 times over basal values at 24 hr after indomethacin treatment (Table 2). Either L-NAME or aminoguanidine significantly decreased the NO production in the mucosa in response to indomethacin. Although a small amount of NO production was observed in normal rats without indomethacin treatment, this NO production was significantly reduced by L-NAME but not aminoguanidine.

**Myeloperoxidase (MPO) activity**

The MPO activity representing the neutrophil infiltration in the mucosa was markedly elevated from about 6 hr after administration of indomethacin. Neither SC-560 nor rofecoxib alone increased MPO activity in the intestinal mucosa, yet the combined administration of these two agents significantly increased the MPO activity as compared with control values observed in normal rats. The increase in MPO activity in response to indomethacin was significantly suppressed by treatment of the animals with dmPGE$_2$ and ampicillin as well as atropine. Aminoguanidine also significantly prevented an increase in MPO activity following indomethacin, although too much less of an extent than the other agents (Table 1).

**FUNCTIONAL MECHANISM OF COX-2 EXPRESSION**

SC-560 but not rofecoxib caused the expression of COX-2 mRNA in the intestinal mucosa, similar to conventional NSAIDs. The up-regulation of COX-2 expression by SC-560 was apparently inhibited by prior administration of either dmPGE2, ampicillin or atropine (Fig. 11). Neither of these agents had any effect on the expression of COX-1 as well as G3PDH in the intestinal mucosa. Similar results were obtained when the COX-2 expression was induced by indomethacin, in place of SC-560. On the other hand, SC-560 markedly decreased the mucosal PGE$_2$ content of the small intestine when determined 3 hr after the administration, but this effect disappeared 12 hr later (see Fig. 5). The mucosal PGE$_2$ content observed at 12 hr after administration of SC-560 was significantly decreased by the co-administration of rofecoxib. Similarly, this PGE$_2$ recovery seen after SC-560 was also significantly prevented when the animals were pretreated with either ampicillin or atropine at doses that inhibited the up-regulation of COX-2 expression (Fig. 12).
The intestinal ulcerogenic property of NSAID is not accounted for solely by COX-1 inhibition and requires the inhibition of both COX-1 and COX-2 (22, 23). This contention is supported by the finding that the selective COX-1 nor COX-2 inhibitor alone caused gross damage in the small intestine, but the combined administration of these two inhibitors provoked intestinal lesions, similar to indomethacin the nonselective COX inhibitor. Inhibition of COX-1 up-regulated the COX-2 expression in the intestinal mucosa, which may explain for the lack of gastric ulcerogenic property of the selective COX-1 inhibitor. We also confirmed an increase of intestinal motility, enterobacterial invasion, and the increase in iNOS as well as MPO activity following administration of indomethacin (8, 9, 25), and showed that the former two events were due to COX-1 inhibition, but the increase of iNOS and MPO activity occurred only when both COX-1 and COX-2 were inhibited (23).

First, the conventional NSAIDs (nonselective COX inhibitors) such as indomethacin, naproxen, flurbiprofen or diclofenac produced damage in the small intestine with a marked decrease of the mucosal PGE\(_2\) contents, confirming a PG deficiency in the background for NSAID-induced intestinal lesions (4, 10). However, the selective COX-1 inhibitor SC-560 did not produce any gross damage, despite inhibiting PG production and decreasing PGE\(_2\) contents in the intestinal mucosa (22). On the other hand, the selective COX-2 inhibitor rofecoxib did not affect on the mucosal PG production and induce any damage. Of interest, the combined administration of SC-560 plus rofecoxib, however, provoked damage in the small intestine, in a dose-dependent manner (22). These data argue the contention that COX-1 but not COX-2 plays a role in maintaining the mucosal...

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**Fig. 11.** Effects of various drugs on the expression of COX-2 after administration of SC-560 in the rat small intestine. The animals were administered SC-560 (SC: 10 mg/kg) p.o., and killed 6 hr later. DMPGE\(_2\) (30 µg/kg) and atropine (3 mg/kg) were given s.c. 30 min before indomethacin while ampicillin (800 mg/kg) was given p.o. twice 24 hr and 30 min before indomethacin. M: marker, Cont: control, V: vehicle, Amp: ampicillin, Atr: atropine. (Unpublished data).
integrity of the small intestine, and strongly suggest that inhibition of both COX-1 and COX-2 is required for the occurrence of NSAID-induced intestinal damage.

Secondly, we clearly showed the role of COX-1 and COX-2 inhibition in a variety of pathogenic events responsible for NSAID-induced intestinal damage, including intestinal hypermotility, bacterial invasion and an increase of MPO as well as iNOS activity (23). Of interest, SC-560 also caused an increase in intestinal motility and bacterial number in the mucosa, suggesting a role for COX-1 inhibition in intestinal hypermotility in response to NSAIDs as well as a causal relationship between the hypermotility and enterobacterial invasion. Indeed, it has been shown that atropine, an anticholinergic drug, inhibits intestinal hypermotility induced by indomethacin, resulting in suppression of bacterial invasion and other inflammatory changes in the small intestine (10, 24). Boughton-Smith et al. (25) reported that bacterial endotoxin enhances the intestinal permeability through expression of iNOS and overproduction of NO in the mucosa. This result is supported by the present finding that indomethacin up-regulated iNOS expression with concomitant increase in iNOS activity. The expression of iNOS mRNA was also observed in the intestinal mucosa following SC-560 but not rofecoxib, indicating that the up-regulation of iNOS is associated with the inhibition of COX-1 (23). This is understandable, because iNOS expression is triggered by endotoxin released from enterobacteria (25), and because bacterial invasion is causally related with intestinal hypermotility due to COX-1 inhibition (24). This idea was supported by the finding that the up-regulation of iNOS mRNA following

Fig. 12. Effects of various drugs on the mucosal PGE₂ content of the rat small intestine at 12 hr after administration of SC-560. The animals were administered SC-560 (10 mg/kg) p.o., and killed 12 hr later. Atropine (3 mg/kg) was given s.c. twice 30 min before and 9 hr after SC-560 while ampicillin (800 mg/kg) was given p.o. twice 24 hr and 30 min before SC-560. Rofecoxib (10 mg/kg) was given p.o. as a single injection together with SC-560. Data are presented as the mean±SE from 6 rats. *Significant difference from vehicle, at p<0.05. (Unpublished data).
indomethacin was attenuated in the presence of ampicillin as well as dmPGE₂ (26). However, SC-560 did not increase the iNOS activity in the mucosa, despite up-regulating iNOS mRNA expression, and a significant increase in this activity was observed when SC-560 was given together with rofecoxib. Since the severity of indomethacin-induced intestinal damage was significantly reduced by aminoguanidine the relatively selective iNOS inhibitor as well as 16,16-dimethyl PGE₂ given 6 hr after administration of indomethacin (8, 22), it is assumed that PGE₂ may inhibit the iNOS activity similar to aminoguanidine. Alternatively, it is also possible that PGE₂ regulates the post-transcriptional regulatory mechanisms to decrease the iNOS protein expression or increase the protein degradation.

It is no doubt that endogenous NO produced by iNOS plays a pathogenic role in the occurrence of intestinal ulceration following subcutaneous administration of indomethacin (27). However, the intestinal ulcerogenic response to indomethacin was worsened or prevented by pre- or post-administration of L-NAME a non-selective NOS inhibitor, respectively (8). These results strongly suggest that NO plays a dual role in the pathogenesis of this ulcer model, a protective role by cNOS/NO and a proulcerogenic role by iNOS/NO. We also showed that indomethacin-induced intestinal lesions were significantly prevented by prior administration of NOR-3 the NO donor (28). The protective effect of NO is causally related with suppression of enterobacterial translocation, the process being functionally associated with stimulation of mucus and fluid secretions as well as inhibition of intestinal hypermotility (28, 29). These functional changes may strengthen a barrier against enterobacteria, resulting in prevention of bacterial translocation and inhibition of the iNOS up-regulation, and by so doing prevent the development of small intestinal lesions following indomethacin. Certainly, the blockade of cNOS/NO production by L-NAME causes a decrease of mucus and fluid secretions and an increase of motility, resulting in enhancement of bacterial translocation and aggravation of this lesion model.

Wallace et al. (20) reported that SC-560, but not celecoxib, produced a decrease in gastric mucosal blood flow, suggesting that the effect of NSAIDs on the mucosal blood flow is brought about by suppression of COX-1. This may be compatible with the present finding that intestinal hypermotility was induced by inhibition of COX-1 but not COX-2 activity, because intestinal hypermotility caused mucosal hypoxia and microvascular injury due to smooth muscle contraction (30, 31). It has also been shown that the selective COX-2 inhibitor celecoxib increased neutrophil adherence in mesenteric venules similar to indomethacin, whereas the selective COX-1 inhibitor SC-560 did not (20). The intestinal MPO activity was not affected by SC-560 or rofecoxib alone, but was increased only when both COX-1 and COX-2 were inhibited by the combined administration of SC-560 plus rofecoxib. We previously reported that the increased MPO response to indomethacin was suppressed by ampicillin as well as atropine, suggesting that this event is closely associated with enterobacterial invasion (10, 24, 32). Because inhibition of COX-1 by SC-560 up-regulated
COX-2 expression which in turn increased PGE\textsubscript{2} production from 6 hr after the administration, as a compensatory response to suppression of the biosynthesis of PG by COX-1 inhibition, it is assumed that inhibition of COX-2 may be related with the increase in MPO activity after the combined administration of SC-560 plus rofecoxib. It is known that neutrophils play a permissive role in NSAID-induced intestinal damage, inasmuch as these lesions were significantly prevented by anti-neutrophil serum (9). These blood cells are a source of oxygen radicals and iNOS, and peroxynitrites formed by the interaction of NO with oxygen radicals may be detrimental in this lesion model (33). Thus, it may be assumed that COX-2 contributes to maintaining the integrity of the intestinal mucosa through inhibition of neutrophil migration under inhibition of COX-1.

**Fig. 13.** Working hypothesis on the roles of COX-1 and COX-2 in the pathogenic mechanism of NSAID-induced intestinal damage. NSAIDs cause intestinal hypermotility, followed by enterobacterial invasion, iNOS induction, and neutrophil activation, and by so doing result in intestinal damage. The intestinal hypermotility and subsequent bacterial invasion are associated with a PG deficiency caused by COX-1 inhibition. On the other hand, the inhibition of COX-1 up-regulates COX-2 expression associated with bacterial invasion, and the PGE\textsubscript{2} produced by COX-2 suppresses the subsequent events such as neutrophil activation and prevents the development of damage. This sequence of events related to COX-1 or COX-2 inhibition may explain why intestinal damage occurs only when both COX-1 and COX-2 are inhibited.
On the other hand, the up-regulation of COX-2 expression following indomethacin was attenuated by atropine at a dose that inhibited the intestinal hypermotility response following indomethacin, suggesting a relationship between the hypermotility and COX-2 expression. This idea is supported by the finding that both events were observed after administration of SC-560, the selective COX-1 inhibitor. Since inhibition of the intestinal hypermotility resulted in suppression of bacterial invasion in the mucosa (24) and since enterobacteria induce the expression of iNOS through release of endotoxin (25), it is possible that the expression of COX-2 is up-regulated by endotoxin, similar to that of iNOS in the mucosa. As expected, ampicillin prevented the expression of COX-2 seen after the indomethacin treatment, despite having no effect on the intestinal hypermotility response. These results strongly support the idea that the expression of COX-2 under inhibition of COX-1 is closely associated with intestinal hypermotility and subsequent bacterial invasion. We have shown that the up-regulation of iNOS expression following administration of indomethacin or SC-560 was also inhibited by both atropine and ampicillin (26).

Considering the results of the present study together with findings by others, one may speculate that conventional NSAIDs produce intestinal hypermotility (10, 24), followed by bacterial translocation and microvascular disturbances, leading to neutrophil activation and iNOS expression, and by so doing cause intestinal damage (4-8) (Fig. 13). The intestinal hypermotility and bacterial translocation are associated with a deficiency of PG caused by inhibition of COX-1 (23). However, inhibition of COX-1 up-regulates the expression of COX-2, and PGs produced by COX-2 may suppress the detrimental processes associated with COX-1 inhibition, including the increases in MPO and iNOS activity (20, 23, 34). In addition, since ampicillin prevented the expression of COX-2 seen after indomethacin, despite causing no effect on the intestinal hypermotility response (24, 26), it is assumed that the expression of COX-2 under inhibition of COX-1 is closely associated with intestinal hypermotility and subsequently occurred 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. Finally, it is concluded that COX-2 as well as COX-1 play a role in maintaining the mucosal integrity of the small intestine and that inhibition of both COX-1 and COX-2 is required for the intestinal ulcerogenic properties of NSAIDs.

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