

Lansoprazole, a proton pump inhibitor, reduces the severity of indomethacin-induced rat enteritis

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Abstract. The spread of capsule endoscopy has led to a focus on small intestinal injury induced by non-steroidal anti-inflammatory drugs (NSAIDs). However, it has been proposed that proton pump inhibitors (PPI), a strong anti-secretory agent, have anti-inflammatory action beyond acid suppression. Therefore, we evaluated the biological effects of lansoprazole, a PPI used in the clinical area, in the setting of experimental rat non-steroidal anti-inflammatory drug-induced enteritis. The animals were given indomethacin subcutaneously and the intestinal mucosa was examined 24 h later. Lansoprazole was given subcutaneously just after following indomethacin injection. Single administration of indomethacin at 10 mg/kg provoked severe hemorrhagic lesions in the small intestine, mostly the jejunum and ileum. The levels of thiobarbituric acid-reactive substances (TBARS), the myeloperoxidase (MPO) activity and the content of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in the intestinal mucosa significantly increased in indomethacin-treated groups compared with the sham-operated groups. The development of intestinal lesions in response to indomethacin was dose-dependently prevented by lansoprazole at a dose of 5 mg/kg together with significant suppression of the increased level of TBARS, MPO activities and CINC-1 in the small bowel. Furthermore, the increased CINC-1 mRNA expression after administration of indomethacin was also inhibited by treatment with lansoprazole. These results suggest that lansoprazole administered exogenously prevented the small intestine against indomethacin-induced damage, the action being dependent on its anti-inflammatory and anti-oxidative responses. This evidence

supports the theory that PPI have an expanding role beyond acid suppression.

Introduction

The anti-inflammatory, analgesic, and anti-pyretic properties of non-steroidal anti-inflammatory drugs (NSAIDs) are particularly useful in treating rheumatic and other musculoskeletal disorders. The clinical utility of an NSAID is determined by the compromise between its therapeutic efficacy and toxicity.

A major limitation of the clinical utility of NSAIDs is their gastroduodenal epithelial toxicity. The first reports of NSAID toxicity in the small bowel were from laboratory animal studies demonstrating indomethacin-induced intestinal bleeding, inflammation, and ulceration (1).

It is now well known that NSAIDs, such as indomethacin and aspirin, cause damage in the small intestines and stomachs of humans and experimental animals as an adverse effect (2-4). Intestinal injury caused by indo-methacin is associated with increased mucosal permeability, microvascular injury, focal intravascular thrombus formation, fibrin deposition, and neutrophil infiltration. Ulcerations and adhesions are also prominent features of this injury. Several factors have been postulated as the pathogenic element of indomethacin-induced intestinal lesions, including bacterial translocation, neutrophils and nitric oxide (NO) overproduction, in addition to prostaglandin (PG) deficiency (5-9).

Proton pump inhibitors (PPI) (10), such as omeprazole and lansoprazole, strong anti-secretory agents that act on gastric (H⁺/K⁺) ATPase of parietal cells (11), have been extensively used for therapeutic control of acid-related disorders, including gastroesophageal reflux disease and peptic-ulcer diseases caused by stress, non-steroidal anti-inflammatory drugs and *Helicobacter pylori* infection (12-15).

Recently, it has been suggested that PPI inhibit neutrophil activation, such as chemotaxis, superoxide production and degradation (16). We have already reported that PPI can attenuate neutrophil adherence to endothelial cells via inhibition of the expression of adhesion molecules (17) and that lansoprazole can ameliorate acid-unrelated intestinal mucosal damage induced by ischemia-reperfusion in rats (18).

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These results indicated that PPI have anti-inflammatory effects as well as inhibition of acid secretion.

Given the above background, the purpose of the present study was to evaluate the protective effects of lansoprazole, on indomethacin-induced enteritis in rats.

Materials and methods

Experimental animals. Male Wistar rats weighing 190-210 g were obtained from Kearsy Co., Ltd. (Osaka, Japan). The rats were housed in stainless steel cages with wire bottoms and maintained on a 12-h light and 12-h dark cycle, with the temperature and relative humidity of the animal room being controlled at 21-23°C and 55-65%, respectively. The experiments were performed using 5-6 non-fasting rats per group under unanesthetized conditions, unless otherwise specified. All experimental procedures described below were approved by the Animal Care Committee of the Kyoto Prefectural University of Medicine (Kyoto, Japan).

Induction of small intestinal lesions. The animals were subcutaneously administered indomethacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid; Sigma Chemical, St. Louis, MO) (10 mg/kg) and killed 6, 12 and 24 h later under deep ether anesthesia, and both the jejunum and ileum were removed. Then, they were opened along the anti-mesenteric attachment and examined for lesions under a dissecting microscope with square grids. The area (mm²) of visible lesions was macroscopically measured, totalled per 15 cm of small intestine, and expressed as a lesion score. The person measuring the lesions did not know the treatments given to the animals. Lansoprazole, a gift from Takeda Chemical Industries Ltd., Japan, was given subcutaneously (1-5 mg/kg) just after indomethacin injection. Both indomethacin and lansoprazole were suspended in 0.5% of carboxymethylcellulose solution with a drop of Tween-80.

Assessment of intestinal mucosal injury induced by indomethacin. To estimate the severity of the intestinal mucosal damage by indomethacin, the concentrations of thiobarbituric acid-reactive substances (TBARS) in the intestinal mucosa, an index of lipid peroxidation, were measured by the method of Ohkawa *et al* (19). In brief, the small intestine was opened by a longitudinal incision, and the intestinal mucosa was scraped off using two glass slides. Mucosal tissue was then homogenized with 10 mM potassium phosphate buffer (pH 7.8) containing 30 mM KCl using a Teflon Potter-Elvehjem homogenizer. The levels of TBARS in the mucosal homogenates were expressed as nmol of malondialdehyde/g wet weight using 1,1,3,3-tetramethoxypropane as the standard.

Myeloperoxidase (MPO) activity in the intestinal mucosa, an index of polymorphonuclear leukocyte accumulation, was determined by a modification of the method of Grisham *et al* (20). Two milliliters of mucosal homogenate was centrifuged at 20,000 x g for 15 min at 4°C to pellet the insoluble cellular debris. The pellet was then rehomogenized in an equivalent volume of 0.05 M potassium phosphate buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide. Samples were centrifuged at 20,000 x g for 15 min at 4°C, and the supernatants were saved. MPO activity was assessed

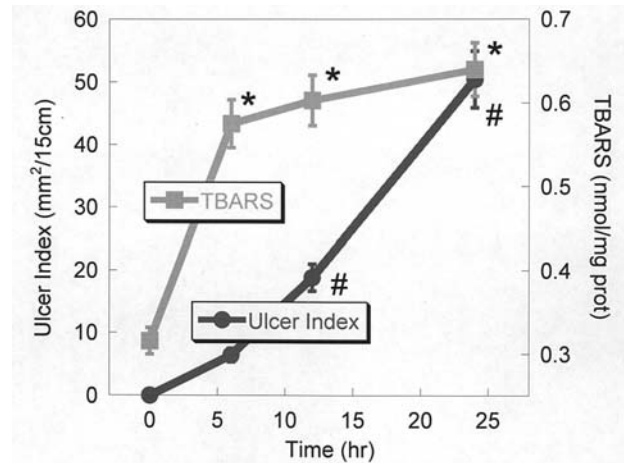


Figure 1. Time course of ulcer index and the level of thiobarbituric acid-reactive substances (TBARS) in intestinal mucosa treated with indomethacin. Indomethacin at a dose of 10 mg/kg was suspended in 0.5% of carboxymethylcellulose solution and was given to rats subcutaneously. Data are expressed as means \pm SEM. * $p < 0.05$ and # $p < 0.05$ versus the levels of 0 h.

by measuring the H₂O₂-dependent oxidation of 3,3',5,5'-tetramethylbenzidine. One unit of enzyme activity was defined as the amount of MPO that caused a change in the absorbance of 1.0/min at 655 nm and 25°C.

The content of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in the intestinal mucosal homogenates was determined by enzyme-linked immunosorbent assay (ELISA) using a kit (Immuno Biological Laboratories, Gunma, Japan) according to the manufacturer's instructions. The absorbance of each well was read at 490 nm by a microplate reader (MPR-A4i; Tosoh, Tokyo, Japan).

The expression of intestinal CINC-1 was determined using the reverse transcription-polymerase chain reaction (RT-PCR) method. Samples of RNA isolation were prepared from the intestinal mucosa. Total RNA was isolated using the acid guanidium phenol chloroform (AGPC) method with Isogen (Nippon Gene). The concentration of RNA was determined by the absorbance at 260 nm in relation to that at 280 nm. The RNA was stored at -70°C until used for RT-PCR. One microliter of RT product was added to 3 mM of each primer, CINC-1 and β -actin (for internal standard purpose), and a solution of 0.5 U of TaqDNA polymerase (Takara Biochemicals) in a final volume of 50 μ l. The primers had the following sequences: for CINC-1, sense 5'-ACAGTGGCAG GGATTCATT-3', and antisense 5'-CTAGCACAGTGGTT GACACT-3'; and for β -actin, sense 5'-TCCTGTGGCATCC ATGAAACT-3', and antisense 5'-GAAGCATTTGCGGTGC ACGAT-3'. The mixture was subjected to 30 cycles (1 min at 94°C, 1 min at 54°C, 1 min at 72°C) of amplification, after which the reaction products were separated by electrophoresis on 2.5% agarose gel and stained with ethidium bromide.

Statistical analysis. All values are expressed as means \pm SEM. The data were compared by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. All analyses were performed using the StatView 5.0-J program (Abacus Concepts Inc., Berkeley, CA). A probability value <5% was considered statistically significant.

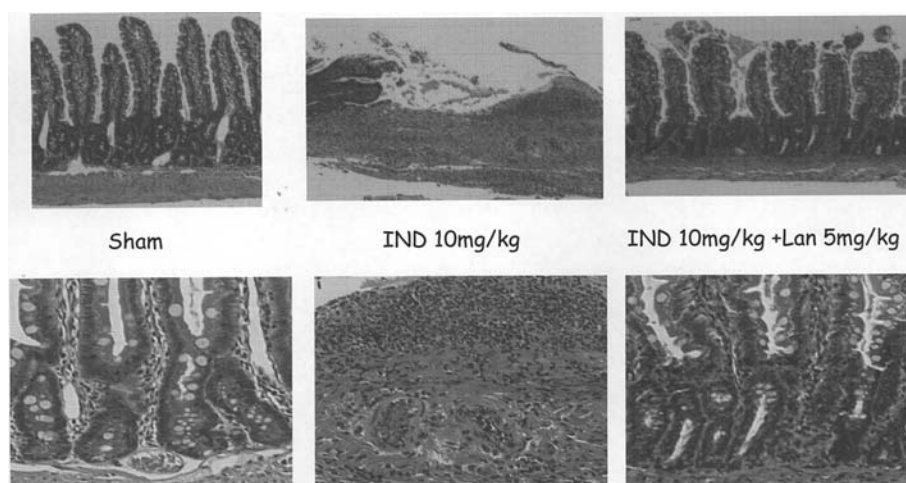


Figure 2. Higher power magnification of histological sections from small intestine of indomethacin-treated rat administered with either vehicle or lansoprazole (5 mg/kg).

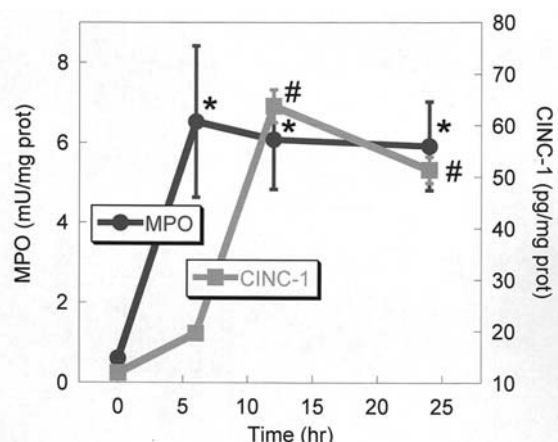


Figure 3. Time course of mucosal myeloperoxidase (MPO) activities and the content of cytokine-induced neutrophil chemoattractant-1 (CINC-1) in intestinal mucosa treated with indomethacin. Indomethacin was administered to rats in the same manner as described in Fig. 1. Data are expressed as means \pm SEM. * $p < 0.05$ and # $p < 0.05$ versus the levels of 0 h.

Results

Histological findings and time course of ulcer index. Single administration of indomethacin at a dose of 10 mg/kg provoked severe hemorrhagic lesions in the small intestine, mostly the jejunum and ileum. The lesion score gradually increased time dependently and showed a significant increase in ulcer index at the time of 12 and 24 h after administration of indomethacin, compared with sham-operated groups (Fig. 1).

The histological features were defect of the villi, epithelial stratification, basal lamina degeneration, and infiltration with neutrophils (Fig. 2).

Time course of the levels of mucosal TBARS, MPO activities and CINC-1. TBA-reactive substances in the intestinal mucosa significantly increased 6, 12 and 24 h after administration of indomethacin (Fig. 1). The MPO activity of intestinal mucosa significantly increased 6 h after administration of indomethacin and showed almost the same pattern as the change of TBARS

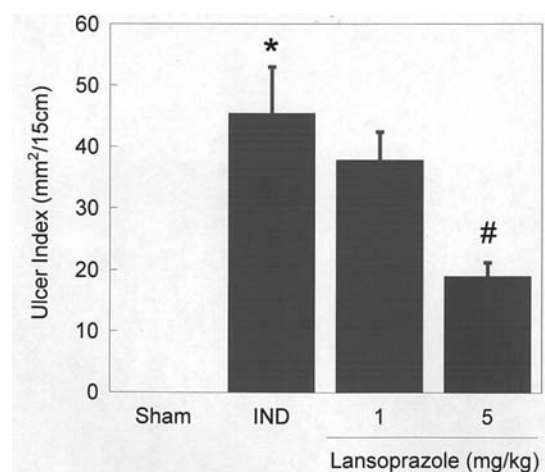


Figure 4. Effect of lansoprazole on ulcer index in intestinal mucosa treated with indomethacin. Lansoprazole was suspended in 0.5% of carboxymethyl-cellulose solution and was given subcutaneously (1-5 mg/kg) just after indomethacin injection. Rats in the sham-operated group and the indomethacin-treated group without lansoprazole received an equivalent volume of vehicle instead of lansoprazole. Data are expressed as means \pm SEM. * $p < 0.05$ versus sham-operated group; # $p < 0.05$ versus the indomethacin-treated group without lansoprazole.

(Fig. 3). The content of mucosal CINC-1 in the indomethacin groups gradually increased but showed significant levels at 12 and 24 h after indomethacin administration, compared with levels in the sham-operated groups (Fig. 3).

The effects of lansoprazole on ulcer index. The development of intestinal lesions in response to indomethacin was dose-dependently prevented by lansoprazole (1-5 mg/kg) 24 h after administration of indomethacin, and a significant effect was observed at over 5 mg/kg (Figs. 2 and 4).

The effects of lansoprazole on mucosal levels of TBARS, MPO activities and CINC-1. TBA-reactive substances in the intestinal mucosa significantly increased 24 h after indomethacin administration and these levels were significantly decreased by lansoprazole treatment at a dose of 5 mg/kg (Table I).

Table I. Effect of lansoprazole on TBARS, MPO activities and CINC-1 levels of intestinal mucosa 24 h after administration of indomethacin (10 mg/kg, s.c.).

	Sham		Indomethacin (10 mg/kg)	
	Lansoprazole (-)	Lansoprazole (+)	Lansoprazole (-)	Lansoprazole (+)
TBARS (nmol/mg protein)	4.05±1.42	2.67±0.71	7.48±0.49 ^a	4.09±0.65 ^b
MPO (mU/mg protein)	11.2±3.70	9.60±3.30	45.6±10.4 ^a	22.9±7.40 ^b
CINC-1 (pg/mg protein)	24.9±5.00	28.6±12.4	76.7±18.3 ^a	32.0±7.70 ^b

Each value indicates the mean ± SE. ^ap<0.05 compared with the value of sham-operated groups, ^bp<0.05 compared with the value of control (indomethacin without lansoprazole) group.

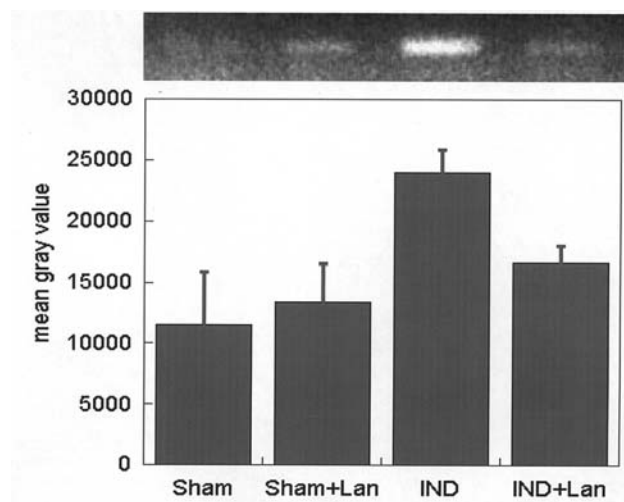


Figure 5. The effect of lansoprazole on CINC-1 mRNA expression in intestinal mucosa 3 h after indomethacin administration in rats. Lansoprazole was administered to rats in the same manner as described in Fig. 4. The CINC-1 expression was determined by reverse transcription-polymerase chain reaction and the figure is representative of at least 3 experiments performed on different days.

The indomethacin group showed a significant increase in mucosal MPO activity compared with the sham-operated group. However, this increase in MPO activity was significantly decreased by treatment with lansoprazole at a dose of 5 mg/kg (Table I).

The content of mucosal CINC-1 in the indomethacin groups was significantly increased compared with levels in the sham-operated groups. This increase in the levels of inflammatory cytokine was significantly inhibited by treatment with lansoprazole at a dose of 5 mg/kg (Table I).

Expression of intestinal CINC-1 mRNA after administration of indomethacin. The expression of CINC-1 mRNA in the intestinal mucosa was up-regulated by the subcutaneous administration of indomethacin. This increase in the expression of CINC-1 mRNA was also inhibited by treatment with lansoprazole at a dose of 5 mg/kg (Fig. 5).


Discussion

Our results clearly show that lansoprazole administered exogenously had a prophylactic effect against intestinal

ulcerogenic response induced by indomethacin. In addition, increases in TBARS contents, MPO activity and CINC-1 content were inhibited by the treatment of lansoprazole. The present study demonstrated for the first time that lansoprazole prevented the development of small intestinal lesions following administration of indomethacin.

Many studies using animal models have suggested that the pathogenesis of indomethacin-induced small intestinal toxicity probably involves multiple interactions dependent on enterohepatic recirculation, prostaglandin depletion, epithelial permeability, neutrophil infiltration, nitric oxide and bacterial infection (5-9). With regard to the active oxygen species in the pathogenesis of indomethacin-induced enteritis, evidence of oxidative stress was found in the mucosa of the small intestine of rats administered indomethacin, as indicated by the increased activity of xanthine oxidase with a corresponding decrease in the levels of several free radical scavenging enzymes (21). On the other hand, it has been reported that indomethacin induced mitochondrial dysfunction and oxidative stress in villus enterocytes (22). Furthermore, Davies and Jamali showed the protective effects of tempo, such as free radical scavenger, against indomethacin-induced intestinal mucosal damage, suggesting that free radicals may be important mediators in the pathogenesis of this enteritis (23).

Little is known about the anti-inflammatory and anti-oxidative mechanisms of lansoprazole against indomethacin-induced intestinal mucosal injury. However, there have been some reports about the anti-inflammatory action of PPI. We have demonstrated that the expression of adhesion molecules on neutrophils and endothelial cells, elicited by *H. pylori* and IL-1 β respectively, is inhibited by lansoprazole and omeprazole at clinically relevant doses (17). Other researchers have also reported that PPI can prevent the neutrophil-endothelial cell adhesion reaction via inhibition of the expression of adhesion molecules (24,25). Some reports regarding the anti-oxidative effects of PPI have also appeared. Suzuki *et al* have reported that PPI inhibit the production of oxygen-derived free radicals from neutrophils activated by chemotactic peptides or opsonized zymosan (26). Other studies have concluded that PPI block stress-induced increase in reactive oxygen intermediates followed by lipid peroxidation and protein oxidation, indicating that their antioxidant properties play a major role in preventing oxidative damage (27-29). Furthermore, our recent study revealed that lansoprazole can ameliorate acid-unrelated intestinal mucosal damage induced by ischemia-reperfusion in rats (18).

 SPANDIDOS PUBLICATIONS lansoprazole has been widely used for the treatment of

acid diseases, including reflux esophagitis. This drug is thought to be transformed into two active species which inhibit acid secretion by (H⁺, K⁺)-ATPase within the parietal cell canaliculus. However, acid secretion was not directly involved in the pathogenesis of the indomethacin-induced intestinal injury model that we used. In the present study, lansoprazole was found to prevent lipid peroxidation and to reduce the development of intestinal mucosal inflammation via inhibition of the production of inflammatory cytokines such as CINC-1. These results suggest that lansoprazole may protect against indomethacin-induced mucosal injury via an anti-inflammatory effect but not inhibition of acid secretion.

It was previously reported that CINC-1 was related with the extent of mucosal damage (30). It is proposed that CINC can be produced by many types of cells, including macrophages, monocytes, and endothelial cells (31). In the present study, lansoprazole inhibited both CINC-1 protein and mRNA in the small intestine after administration of indomethacin. These results suggest that lansoprazole may prevent CINC-1 production by scavenging active oxygen species that are related to signal transduction for the promotion of CINC-1 synthesis. Further study is necessary in order to determine the molecular mechanisms involved in the inhibition of CINC-1 production by lansoprazole.

Collectively, these observations clearly illustrate that lansoprazole protects against acid-unrelated intestinal injury induced by indomethacin via inhibition of neutrophil-dependent inflammation, suggesting that lansoprazole has potential as a new therapeutic agent for NSAID-induced enteritis as well as gastric injury. In the future, clinical and scientific research should go beyond the examination of acid suppression.

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