ORIGINAL ARTICLE

Protective effects of proton pump inhibitors against indomethacin-induced lesions in the rat small intestine

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Abstract Proton pump inhibitors (PPIs) have been shown to be effective in preventing gastric and duodenal ulcers in high-risk patients taking nonsteroidal anti-inflammatory drugs (NSAIDs); by contrast, scarce information is available concerning the effects of PPIs on intestinal damage induced by NSAIDs in humans or in experimental animals. We examined the effects of lansoprazole and omeprazole on the intestinal injury induced by indomethacin in the conscious rat. PPIs were administered by the intragastric route at 30, 60 and 90 μmol/kg, 12 h and 30 min before and 6 h after indomethacin treatment. The effects of omeprazole and lansoprazole were evaluated on: (1) macroscopic and histologic damage; (2) mucosal polymorphonuclear cell infiltration; (3) oxidative tissue damage and (4) bacterial translocation from lumen into the intestinal mucosa. Lansoprazole and omeprazole (at 90 μmol/kg) significantly decreased ($P<0.01$) the macroscopic and histologic damage induced by indomethacin in the rat small intestine. Furthermore, both drugs greatly reduced ($P<0.01$) the associated increases in myeloperoxidase levels and lipid peroxidation induced by indomethacin, whereas they only moderately affected ($P<0.05$) the translocation of enterobacteria from lumen into the intestinal mucosa. These data demonstrate that omeprazole and lansoprazole can protect the small intestine from the damage induced by indomethacin in the conscious rat. The intestinal protection, possibly related to antioxidant and anti-inflammatory properties of these drugs, may suggest new therapeutic uses of PPIs in intestinal inflammatory diseases.

Keywords Proton pump inhibitors · Indomethacin · Rat · Small intestine

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Abbreviations

CD	Crohn's disease
CFU	colony-forming unit
CMC	carboxymethylcellulose
COX	cyclooxygenase
HTAB	hexadecyl trimethyl ammonium bromide
IBD	inflammatory bowel disease
MDA	malondialdheyde
MPO	myeloperoxidase
NSAID	nonsteroidal anti-inflammatory drug
PG	prostaglandin
PPI	proton pump inhibitor
TBARS	thiobarbituric acid reactive substances
TSA	tryptone soya agar
UC	ulcerative colitis

Introduction

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with the development of upper gastrointestinal tract mucosal injury, particularly in high-risk patients (Wolfe et al. 1999). It is now well recognized that these drugs, besides causing gastric ulceration, may damage the small intestine and the colon in healthy subjects and exacerbate disease activity both in ulcerative colitis (UC) and Crohn's disease (CD), leading to small bowel perforation, bleeding and strictures (Bjarnason et al. 1993; Fortun and Hawkey 2005; Thiéfin and Beaugerie 2005). The etiology of NSAID damage to the bowel is complex and still incompletely understood; cyclooxygenase (COX) inhibition appears to have a minor role in the pathogenesis of NSAID enteropathy, although the protective effects of misoprostol suggest that prostaglandin (PG) depletion is partly involved (Whittle 2004). Three different crucial steps seem to be involved in the pathogenesis of NSAID enteropathy (Beck et al. 1990; Reuter et al. 1997; Konaka et al. 1999; Whittle 2004): (1) uncoupling of mitochondrial oxidative phosphorylation which leads to increased intestinal permeability, (2) aggression of the mucosa by bile and luminal bacteria and (3) neutrophil chemotaxis and activation. As observed in many other gastrointestinal inflammatory conditions (Wallace et al. 1990; Halliwell et al. 2000), a crucial role has been attributed to the oxidative stress and the production of reactive oxygen species from activated neutrophils that invade the intestinal mucosa (Yamada and Grisham 1991; Somasundaram et al. 2000; Basivireddy et al. 2002). Finally, the enterohepatic re-circulation of NSAID molecule has been considered a contributing factor in enhancing topical mucosal injury, by repeatedly exposing the intestinal mucosa to high NSAID levels (Reuter et al. 1997; Whittle 2004).

Whereas much attention has been directed toward the prevention/treatment of gastroduodenal ulcers during chronic NSAID use, no drug is currently recommended for the therapy of NSAID-induced intestinal damage (Laine 2001; Goldstein 2004; Fortun and Hawkey 2005). Clinical trials with misoprostol gave inconsistent results and the use of antibiotics, based on the pathogenetic role of bacteria, was unsuccessful (Fortun and Hawkey 2005). Finally, the possibility of circumventing NSAID-related injury by using selective COX-2 inhibitors is still under evaluation (Thiéfin and Beaugerie 2005).

Proton pump inhibitors (PPIs) such as omeprazole and lansoprazole, are the drugs of choice for the treatment of acid-related disorders (Robinson 2004), their high clinical efficacy being due to the blockade of the enzyme H^+/K^+-ATPase (the gastric proton pump), which is the last step in the acid-secretory process (Hersey and Sachs 1995). Several double-blind randomized trials have clearly docu-mented that PPIs are as effective as misoprostol (Hawkey et al. 1998; Graham et al. 2002), and superior to histamine H_2-receptor antagonists (Yeomans et al. 1998; Lai et al. 2003) in preventing NSAID-associated gastric and duodenal ulcers. Therefore, because of the lower compliance and the poor tolerability profile of misoprostol, current treatment guidelines recommend the use of PPIs as a strategy of choice in high-risk patients taking NSAIDs; actually, a co-therapy with a PPI is suggested also in patients treated with selective COX-2 inhibitors, although originally considered safer than traditional NSAIDs (Laine et al. 2003).

It has been recently postulated that the antiulcer and gastroprotective effects of PPIs may also involve acid-unrelated mechanisms, that include production of mucosal protective factors (Takahashi et al. 1997; Tsuji et al. 2002), inhibition of neutrophil infiltration and of oxidative tissue damage (Kobayashi et al. 2002; Biswas et al. 2003; Natale et al. 2004; Blandizzi et al. 2005; Fornai et al. 2005). To support this, several in vitro studies have revealed that omeprazole and lansoprazole possess a direct scavenging activity against oxygen free radicals and inhibit neutrophil function (Wandall 1992; Suzuki 1995; Lapenna et al. 1996; Suzuki et al. 1996; Yoshida et al. 2000; Zedtwitz-Liebenstein et al. 2002; Simon et al. 2006). Thus, it is plausible to hypothesize that the antioxidant properties of PPIs may be beneficial in other pathologic conditions associated with oxidative damage, including NSAID enteropathy or inflammatory bowel disease (IBD; Halliwell et al. 2000; Podolsky 2002; Hanauer 2006). Data concerning intestinal effects of PPIs are few and controversial. Recently, it has been reported that parenteral administration of lansoprazole reduced intestinal mucosal lesions produced by ischemia-reperfusion (Ichikawa et al. 2004) or by indomethacin in rats (Kuroda et al. 2006); on the other hand, in humans, case reports of microscopic or collagenous colitis have been associated with the use of lansoprazole (Thomson et al. 2002; Wilcox and Mattia 2002), and contrasting findings were reported on the ability of omeprazole to induce protective effects on the intestine (Heinzow and Schlegelberger 1994; Goldstein et al. 2005).

Based on these data, the present study was designed to examine the effects of intragastric treatment of two PPIs widely used in clinical practice, namely omeprazole and lansoprazole, on the intestinal damage induced by acute administration of indomethacin in the conscious rat. Indomethacin-induced enteritis has become a commonly used experimental model to unravel new approaches in the prevention and/or therapy of NSAID-induced enteropathy (Yamada et al. 1993; Elson et al. 1995). In addition, this model is extensively employed to clarify the pathogenesis of intestinal inflammation and for the screening of novel

drugs for IBD treatment, due to the histo-pathological and functional similarities between ileal CD and indomethacin jejunal lesions in rats (Anthony et al. 2000).

Materials and methods

Experimental animals

Male Wistar rats (220–240 g) were purchased from Harlan-Italy (Milan, Italy). They were housed in a restricted access room with controlled temperature (23°C) and a light/dark (12 h:12 h) cycle, and allocated in wire mesh cages with a maximum of 4 subjects per cage. Food and water were provided ad libitum. The study received the approval of the local Animal Ethic Committee of the Faculty of Medicine, University of Parma, Italy.

Induction of intestinal damage

Enteritis was induced in separate groups (n=6–8 rats for each group) of unfasted rats, by means of a single intragastric administration of 20 mg/kg indomethacin, suspended in 1% carboxymethylcellulose (CMC), in a volume of 5 ml/kg. Control rats received equivalent volumes of 1% CMC. Indomethacin-treated rats were divided in sub-groups, which received by intragastric gavage either 1% CMC (vehicle-treated rats) or multiple administrations of PPIs at selected times (12 h and 30 min before and 6 h after indomethacin treatment). Lansoprazole or omeprazole were administered at three different doses: 30, 60 and 90 μmol/kg. This range of doses was chosen on the basis of previous works showing an inhibition of acid secretion of 80–100% (Satoh et al. 1989; Seensalu et al. 1990) and a protective activity against NSAID-induced gastric damage in rats by oral administration (Morini et al. 1995; Natale et al. 2004; Blandizzi et al. 2005). Multiple intragastric administrations of PPIs were given to ensure adequate systemic bioavailability, due to the high first pass metabolism of these drugs (Robinson 2004). Separate groups of rats received PPIs alone, administered at the above doses following the same protocol, in order to examine their effects on healthy intestine. Rats were killed by cervical dislocation under deep ether anaesthesia 24 h after indomethacin administration and the intestinal lesions were evaluated.

Macroscopic evaluation of intestinal damage

The small intestine was removed from each animal and the first 20 cm of the proximal region, starting from the pylorus, were discarded. The remaining portion of intestine was divided into 5 cm-segments. Intestinal segments were opened along the antimesenteric border, gently cleaned of fecal content, fixed on a slide and photographed for macroscopic evaluation of damage. Damaged area of each segment was calculated by means of a digital image analysis software (ImageJ, NIH) and summed per small intestine. The ulcerogenic effect of drugs was expressed as a percentage of damaged area over the total examined intestinal mucosa. The examiners were unaware of animal treatment.

Histology

Histology was carried out on segments of small intestine removed from four rats of each group. Intestinal segments were immediately injected with 10% formalin and left in the same fixative solution. After 30 min, they were opened along the anti-mesenteric border, cleaned of fecal content and fixed in 10% formalin for 24 h. From each intestinal segment six sections were randomly chosen and processed into paraffin. Serial paraffin sections (4 μm) were then prepared and stained with hematoxylin-eosin and PAS for morphological examination. Histologic damage was assessed by two observers, blind to the treatment, according to the scoring system by Anthony et al. (1993), modified as follows:

Grade I
– shortening and distortion of villi
– loss of epithelium
– infiltration of epithelium by eosinophils
– no neutrophil infiltration

Grade II
– mucosal neutrophil infiltration
– focal and upper villous necrosis

Grade III
– mucosal and transmural necrosis
– extensive neutrophil infiltration
– floride acute peritonitis

Myeloperoxidase activity

Intestinal myeloperoxidase (MPO) activity was assumed as a quantitative index of mucosal inflammation and was measured according to a previously described method with slight modifications (Bradley et al. 1982). Briefly, a 5 cm-long segment of jejunum from each rat was homogenized in 1 ml hexadecyltrimethyl-ammonium bromide (HTAB) buffer (0.5% in 50 mM phosphate buffer, pH 6.0) for each 50 mg tissue and centrifuged at 12,000 g for 15 min at 4°C. An aliquot of the supernatant (7 μl) from each sample was then added to 200 μl of a reaction mixture containing 0.167 mg/ml *O*-dianisidine dihydrochloride and 0.0005%

hydrogen peroxide in 50 mM phosphate buffer at pH 6.0. Changes in absorbance at 450 nm were measured using a microplate absorbance reader (Tecan Sunrise, Tecan Inc, Mannedorf, Switzerland). One unit of MPO was defined as degrading 1 μmole hydrogen peroxide per minute at 25°C. Data were expressed as units of MPO per mg tissue.

Lipid peroxidation

Membrane lipid peroxidation was determined as an index of oxidative damage and was assessed by measuring thiobarbituric acid reactive substances (TBARS) in intestinal tissues, according to the technique described by Uchiyama and Mihara (1978), with minor modifications. Briefly, samples of jejunum from treated rats were collected, homogenized in 0.15 M KCl (1 ml for 100 mg wet tissue) and centrifuged at 400 g for 10 min. Aliquots (0.5 ml) of supernatants were then mixed to 1 ml 0.6% thiobarbituric acid, 3 ml of 1% phosphoric acid and 83 μl of a 0.2% solution of 2,6-ditert-butyl-4-methylphenol in 95% ethanol. After heating at 85°C for 60 min, samples were then ice-cooled and centrifuged at 2,600 g for 15 min, and the absorbance of the supernatant was measured using a multiplate spectrophotometer (Tecan Sunrise, Tecan Inc, Mannedorf, Switzerland) at a wavelength of 530 nm. Results were expressed as μmoles malondialdehyde (MDA) per mg tissue, MDA representing a suitable index of oxidative tissue damage (Kwiecien et al. 2002).

Mucosal enterobacterial number

Mucosal enterobacterial content was determined, as an indirect index of increased epithelial permeability, according to the method originally described by Reuter et al. (1997), slightly modified. A sample of jejunum of about 10 cm was excised from each rat, opened along the antimesenteric side and gently cleared from fecal content with sterile saline. The sample was then homogenized in 1 ml of sterile Ringer solution per 100 mg wet tissue. Aliquots of the homogenates were then placed on plates containing tryptone soya agar (TSA) or blood agar (Oxoid Ltd, Hampshire, England). TSA plates were incubated at 37°C for 24 h in aerobic conditions, while blood agar plates were incubated for 48 h in an anaerobic environment (Anaerogen, Oxoid Ltd, Hampshire, England). Plates containing 20–200 colony-forming units (CFU) were examined for the number of enteric bacteria and results were expressed as log CFU per gram of tissue.

Statistical analyses

Results are expressed as means±SEM for 6–8 rats. Differences among groups were evaluated by one-way analysis of variance, followed by Dunnett's test. $P < 0.05$ was considered statistically significant. Calculations were performed by commercial software (GraphPad Prism, ver. 3.03, GraphPad Software Inc., San Diego, California, USA).

Drugs

The following drugs and reagents were used: indomethacin and all the analytical chemicals (Sigma Chemicals Co., St. Louis, MO, USA); lansoprazole (Sigma-Tau S.p.A, Pomezia, Italy); omeprazole (Astra Zeneca, Sodertalje, Sweden). All drugs were prepared immediately before use, as suspensions in 1% CMC, and administered by intragastric route in a volume of 5 ml/kg body weight.

Results

Macroscopic damage

Intragastric treatment with 20 mg/kg indomethacin resulted in severe intestinal damage which was characterized by segmental linear mucosal ulcerations extending along the mesenteric border of the jejunum and proximal ileum, bowel thickening and adhesions. The percentage of macroscopically visible damage accounted for 10.2±2.2% of the total examined intestinal area (Fig. 1). Pretreatment with lansoprazole and omeprazole at the lowest dose (30 μmol/kg) did not modify indomethacin-induced injury (data not shown) and induced at 60 μmol/kg a slight inhibition of intestinal lesions, which, however, did not achieve statistical significance (Fig. 1). By contrast, damaged area induced by indomethacin was significantly reduced by lansoprazole
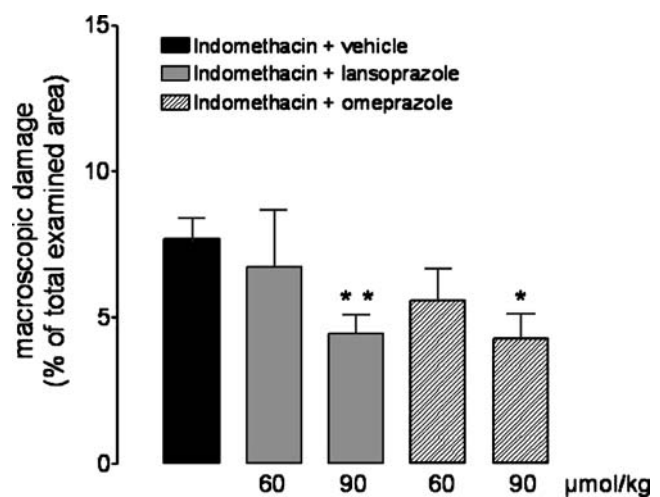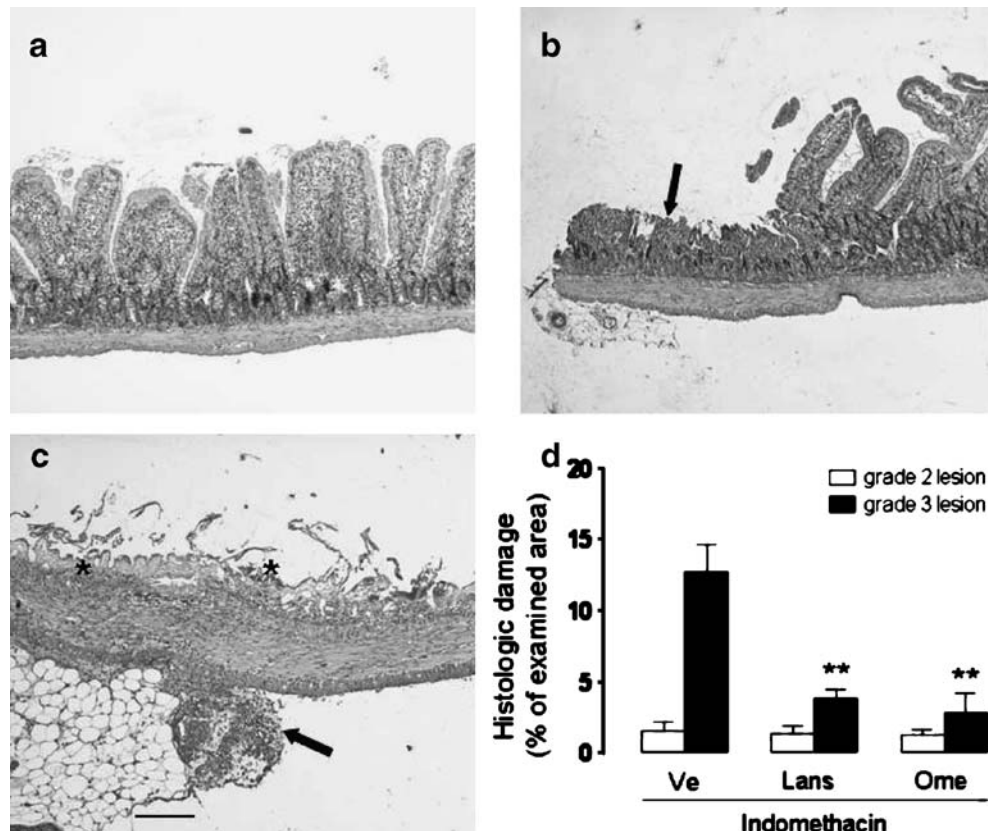

Fig. 1 Macroscopic damage induced by indomethacin (20 mg/kg, intragastrically) in the small intestine of rats pre-treated with intragastric administration of vehicle (1% CMC) or lansoprazole (60 and 90 μmol/kg) or omeprazole (60 and 90 μmol/kg). Bars represent means±SEM. * $P < 0.05$ and ** $P < 0.01$ vs. indomethacin + vehicle

at 90 μmol/kg (4.47±0.66%; $P<0.01$) or by omeprazole at 90 μmol/kg (4.29±0.85%; $P<0.05$; Fig. 1). Therefore, this dose was chosen for the following histologic, biochemical and microbiologic studies. Neither omeprazole nor lansoprazole modified per se the intestinal mucosa (not shown).

Histologic damage

Microscopic examination of the intestinal tissue from rats treated with PPIs alone revealed negligible lesions of surface epithelium, which were similar to those observed in control rats (data not shown). By contrast, in animals treated with 20 mg/kg indomethacin, histology revealed the loss of superficial epithelium, necrosis of the upper villi (grade II lesions, shown in Fig. 2b) and transmural necrosis associated with massive mucosal infiltration by polymorphonuclear cells (grade III lesions, shown in Fig. 2c). Damaged area in these samples extended for 1.56% (grade II lesions) and 12.76% (grade III lesions) of the total examined area (Fig. 2d). Both lansoprazole (90 μmol/kg) and omeprazole (90 μmol/kg) significantly ($P<0.01$) reduced grade III lesions induced by indomethacin (from 12.76 to 3.85 and 2.81%, respectively), while leaving unaffected grade II lesions (Fig. 2d). Overall, PPIs reduced the number of lesions (78 and 70% reduction for omeprazole and lansoprazole, respectively) without influencing the type of lesion.

Myeloperoxidase activity

MPO levels in the intestinal mucosa of control rats were 0.015±0.004 U/mg tissue and were not modified by omeprazole or lansoprazole alone (data not shown). Indomethacin (20 mg/kg) caused a marked increase in mucosal MPO levels (0.052±0.005 U/mg tissue; $P<0.001$) which were significantly ($P<0.05$) reduced by treatment with omeprazole (90 μmol/kg) or lansoprazole (90 μmol/kg) (Fig. 3).

Lipid peroxidation

MDA values in the intestinal mucosa of control rats were 0.021±0.002 μmol/mg tissue and were not modified by PPI treatment (data not shown). Indomethacin (20 mg/kg) significantly increased mucosal MDA levels (0.058± 0.003 μmol/mg tissue; $P<0.01$) (Fig. 3); both lansoprazole (90 μmol/kg) and omeprazole (90 μmol/kg) significantly ($P<0.01$) reduced the tissue accumulation of MDA induced by indomethacin (Fig. 3).

Mucosal bacterial number

The number of aerobic and anaerobic bacteria in the intestinal mucosa of control rats was 5.45±0.33 and 5.64±0.36 log CFU/g tissue, respectively. Indomethacin (20 mg/kg)

Fig. 2 Microscopic aspect of rat jejunum. a Normal architecture of jejunum from control rats. b Grade II lesions induced by indomethacin. Note the focal and upper villous necrosis (arrow). c Grade III lesions induced by indomethacin. Note the transmural necrosis (asterisks), the massive neutrophil infiltration and the acute peritonitis (arrow; Scale bar 250 μm). d Quantitative data of histologic damage in indomethacin-treated rats. Lansoprazole (Lans) and omeprazole (Ome), both administered intragastrically at 90 μmol/kg, significantly decreased grade III lesions induced by indomethacin in comparison with vehicle-treated rats (Ve, 1% CMC). Results are means±SEM. ** $P<0.01$ vs. vehicle
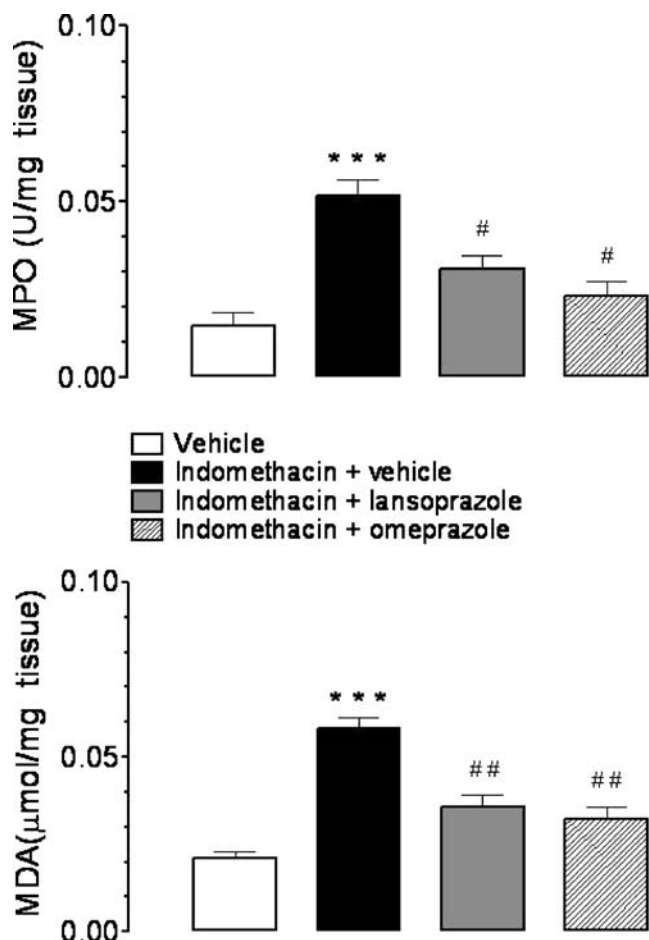
Fig. 3 Evaluation of myeloperoxidase (*MPO*) activity (*top panel*) and malondialdehyde (*MDA*) levels (*lower panel*), as index of neutrophil infiltration and lipid peroxidation, respectively. Intragastric indomethacin (20 mg/kg) induced significant increases in MPO and MDA levels; both values were significantly reduced by lansoprazole (90 μmol/kg) or omeprazole (90 μmol/kg), when compared with vehicle (1% CMC). Results are expressed as means±SEM. #$P<0.05$ and ##$P<0.01$ vs. indomethacin + vehicle; ***$P<0.001$ vs. vehicle

Table 1 Effects of proton pump inhibitors on bacterial translocation in the rat intestinal mucosa induced by indomethacin

Treatment	Enteric bacteria (log CFU/g)	
	Aerobes	Anaerobes
Vehicle	5.45±0.33	5.64±0.36
Indomethacin + vehicle	7.91±0.14[a]	7.87±0.15[a]
Indomethacin + Lansoprazole	7.46±0.13[b]	7.42±0.16[b]
Indomethacin + Omeprazole	7.11±0.30[b]	7.20±0.26[b]

All values are means±SEM obtained from 8 rats
[a] $P<0.01$ vs. vehicle-treated rats
[b] $P<0.05$ vs. indomethacin + vehicle-treated rats

al. 1997; Konaka et al. 1999; Whittle 2004). Topic mucosal injury induced by NSAIDs has been considered of crucial importance in the early phase of intestinal injury, by causing biochemical damage of mitochondria and uncoupling of oxidative phosphorylation (Beck et al. 1990; Somasundaram et al. 2000; Basivireddy et al. 2002); this leads to increased epithelial permeability and exposes the mucosa to luminal aggressive factors such as bile, bacteria and bacterial products (Yamada et al. 1993; Reuter et al. 1997; Konaka et al. 1999; Whittle 2004). The role of luminal bacteria is supported by findings which demonstrated that indomethacin-induced damage is absent or reduced in germ-free rats (Robert and Asano 1997) or after antibacterial treatment (Yamada et al. 1993; Konaka et al. 1999; Kunikata et al. 2002). Neutrophil tissue infiltration is considered a crucial factor in NSAID enteropathy (Yamada and Grisham 1991; Yamada et al. 1993; Whittle 2004). These phagocytic cells may cause tissue injury by producing a variety of oxidants, including superoxide radical and hydrogen peroxide, and by releasing from their cytoplasmic granules an array of damaging enzymes such as myeloperoxidase, elastase and collagenases.

In agreement with previous data (Yamada and Grisham 1991; Yamada et al. 1993; Reuter et al. 1997; Konaka et al. 1999), we observed that indomethacin induced severe intestinal damage in the rat small intestine, that was evident both macroscopically and histologically, resulting in loss of surface epithelium, mucosal necrosis and massive inflammatory cell infiltration. Intestinal lesions were associated with a marked increase in mucosal MPO levels, considered a reliable marker of tissue neutrophil infiltration, together with increased MDA levels, deriving from membrane lipid peroxidation, which is considered a suitable index of oxidative damage. These changes were associated with enhanced bacterial translocation from lumen into the mucosa.

In the present study, we have shown that the intestinal damage induced by indomethacin was prevented by two different proton pump inhibitors, i.e. omeprazole and lansoprazole, administered by intragastric route. The intestinal protection induced by PPIs was evidenced as a

induced a significant ($P<0.01$) increase in mucosal content of both aerobic and anaerobic bacteria, as compared with vehicle-treated rats (Table 1); bacterial translocation induced by indomethacin was significantly ($P<0.05$) decreased by lansoprazole and omeprazole treatment (both at 90 μmol/kg) (Table 1).

Discussion

Intestinal toxicity related to chronic NSAID use is a growing medical problem, particularly in elderly people (Bjarnason et al. 1993; Fortun and Hawkey 2005; Thiéfin and Beaugerie 2005). Pathogenetic factors that have been proposed as initiating events for NSAID enteropathy include both inhibition of protective prostaglandins and COX-independent mechanisms (Beck et al. 1990; Reuter et

significant reduction in the macroscopic damage and was also confirmed histologically by the reduced number of grade III lesions (the more serious lesions induced by indomethacin), that are associated with mucosal necrosis and massive neutrophil infiltration. Likewise, PPIs were able to significantly reduce the increase in MPO levels and lipid peroxidation stimulated by indomethacin. This was also recently observed by Kuroda et al. (2006) with lansoprazole, which was effective after a single subcutaneous treatment with 5 mg/kg. In the present study, both omeprazole and lansoprazole were given by multiple intragastric administrations and induced protective effects at doses that were comparable with those (60–100 μmol/kg) previously reported to be effective in gastric ulceration models (Morini et al. 1995; Natale et al. 2004; Blandizzi et al. 2005) and in acid secretion assays (Satoh et al. 1989; Seensalu et al. 1990).

As for the mechanisms underlying the intestinal protection induced by omeprazole and lansoprazole, it has been previously reported that these drugs can have gastroprotective effects via acid unrelated mechanisms, including inhibition of oxidative tissue damage (Kobayashi et al. 2002; Biswas et al. 2003; Natale et al. 2004; Blandizzi et al. 2005). Free radical production and tissue antioxidant depletion have emerged as common pathogenetic mechanisms in several gastrointestinal diseases (Wallace et al. 1990; Halliwell et al. 2000) and, in particular, seem to play a crucial role in NSAID-induced intestinal mucosal damage (Yamada and Grisham 1991; Somasundaram et al. 2000; Basivireddy et al. 2002).

In this connection, omeprazole was found to have a direct scavenging activity in vitro against hydroxyl radical, one of the major causative factors of gastric ulcer (Lapenna et al. 1996; Biswas et al. 2003); moreover, omeprazole was more potent than naturally occurring antioxidants (i.e. vitamin E) in preventing stress- and indomethacin-induced gastric lesions (Biswas et al. 2003). Antioxidant properties were attributed to the sulphoxide moiety of PPI molecule and were reported also for lansoprazole and pantoprazole (Simon et al. 2006). Besides acting as free radical scavengers, both omeprazole and lansoprazole were found to inhibit oxygen-free radical production from human neutrophils (Suzuki 1995; Suzuki et al. 1996), human neutrophil chemotaxis (Wandall 1992) and adherence to endothelial cells by reducing the expression of neutrophil adhesion molecules (Ohara and Arakawa 1999; Yoshida et al. 2000). Indirect antioxidant effects of PPIs may be related to the inhibition of the proton pump-driven acidification occurring in phagolysosomes of activated polymorphonuclear cells that infiltrate the inflammatory sites (Agastya et al. 2000). The inhibition of V-ATPase of neutrophils, which is crucial for the intracellular pH homeostasis, could lead to reduced neutrophil function

and oxygen free radical generation (Grinstein et al. 1992). In the current study, the inhibition of neutrophil infiltration observed histologically, together with the reduced mucosal MPO levels and lipid peroxidation induced by PPIs support the concept that the main mechanism of PPI-mediated intestinal protection may be related to their anti-inflammatory and antioxidant activity.

Another factor which might contribute to the intestinal protection induced by PPIs could be the reduction of bacterial translocation into the mucosa induced by indomethacin, a process that is considered an early event in determining intestinal damage (Yamada et al. 1993; Reuter et al. 1997; Konaka et al. 1999; Whittle 2004). However, PPI treatment only moderately reduced the number of aerobic and anaerobic bacteria in the intestinal mucosa of indomethacin-treated rats, suggesting that PPI have a minor role in preventing the increase in epithelial permeability and mucosal invasion of luminal enterobacteria.

In summary, data presented herein demonstrate that omeprazole and lansoprazole protect the small intestine against the damaging effect of indomethacin in the conscious rat, their effects being possibly related to the impairment of neutrophil function and to inhibition of oxidative damage. The macroscopic and histologic similarities between jejunal ulceration induced by indomethacin in rats and Crohn's disease in human terminal ileum (Elson et al. 1995; Anthony et al. 2000) might suggest a beneficial effect of omeprazole and lansoprazole in other experimental models of human IBD.

Further studies in humans should determine whether the antioxidant activity and the associated protective effects are a general property of the PPI class and whether these drugs may be a novel therapeutic approach for the prevention of NSAID-induced enteropathy and, more generally, for the control of intestinal inflammation.

References

Agastya G, West BC, Callahan JM (2000) Omeprazole inhibits phagocytosis and acidification of phagolysosomes of normal human neutrophils in vitro. Immunopharmacol Immunotoxicol 22:357–372

Anthony A, Dhillon AP, Nygard G, Hudson M, Piasecki C, Strong P, Trevethick MA, Clayton NM, Jordan CC, Pounder RE, Wakefield AJ (1993) Early histological features of small intestinal injury induced by indomethacin. Aliment Pharmacol Ther 7:29–39

Anthony A, Pounder RE, Dhillon AP, Wakefield AJ (2000) Similarities between ileal Crohn's disease and indomethacin experimental jejunal ulcers in the rat. Aliment Pharmacol Ther 14:241–245

Basivireddy J, Vasudevan A, Jacob M, Balasubramanian KA (2002) Indomethacin-induced mitochondrial dysfunction and oxidative stress in villus enterocytes. Biochem Pharmacol 64:339–349

Beck WS, Schneider HT, Dietzel K, Neurnberg B, Brune K (1990) Gastrointestinal ulcerations induced by anti-inflammatory drugs in rats: physicochemical and biochemical factors involved. Arch Toxicol 64:210–217

Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK (2003) A novel antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of hydroxyl radical. J Biol Chem 278:10993–11001

Bjarnason I, Hayllar J, MacPherson AJ, Russell AS (1993) Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. Gastroenterology 104:1832–1847

Blandizzi C, Fornai M, Colucci R, Natale G, Lubrano V, Vassalle C, Antonioli L, Lazzeri G, Del Tacca M (2005) Lansoprazole prevents experimental gastric injury induced by non-steroidal anti-inflammatory drugs through a reduction of mucosal oxidative damage. World J Gastroenterol 11:4052–4060

Bradley PP, Priebat DA, Christensen RD, Rothstein G (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78:206–209

Elson CO, Sartor RB, Tennyson GS, Riddell RH (1995) Experimental models of inflammatory bowel disease. Gastroenterology 109:1344–1367

Fornai M, Natale G, Colucci R, Tuccori M, Carazzina G, Antonioli L, Baldi S, Lubrano V, Abramo A, Blandizzi C, Del Tacca M (2005) Mechanisms of protection by pantoprazole against NSAID-induced gastric mucosal damage. Naunyn Schmiedebergs Arch Pharmacol 372:79–87

Fortun PJ, Hawkey CJ (2005) Nonsteroidal anti-inflammatory drugs and the small intestine. Curr Opin Gastroenterol 21:169–175

Goldstein JL (2004) Challenger in managing NSAID-associated gastrointestinal tract injury. Digestion 69(Suppl 1):25–33

Goldstein JL, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG, Investigators (2005) Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole and placebo. Clin Gastroenterol Hepatol 3:133–141

Graham DY, Agrawal NM, Campbell DR, Haber MM, Collis C, Lukasik NL, Huang B (2002) Ulcer prevention in long-term users of nonsteroidal anti-inflammatory drugs: results of a double-blind, randomized, multicenter, active- and placebo-controlled study of misoprostol vs. lansoprazole. Arch Int Med 162:169–175

Grinstein S, Nanda A, Lukacs G, Rotstein O (1992) V-ATPases in phagocytic cells. J Exp Biol 172:179–192

Halliwell B, Zhao K, Whiteman M (2000) The gastrointestinal tract: a major site of antioxidant action? Free Rad Res 33:819–830

Hanauer SB (2006) Inflammatory bowel disease: epidemiology, pathogenesis and therapeutic opportunities. Inflamm Bowel Dis 12(Suppl 1):S3–S9

Hawkey CJ, Karrasch JA, Szczepanski L, Walker DG, Barkun A, Swannell AJ, Yeomans ND (1998) Omeprazole compared with misoprostol for ulcers associated with nonsteroidal anti-inflammatory drugs: Omeprazole versus Misoprostol for NSAID-induced Ulcer Management (OMNIUM) Study Group. N Engl J Med 338:727–734

Heinzow U, Schlegelberger T (1994) Omeprazole in ulcerative colitis. Lancet 343:477

Hersey SJ, Sachs G (1995) Gastric acid secretion. Physiol Rev 75:155–189

Ichikawa H, Yoshida N, Takagi T, Tomatsuri N, Katada K, Isozaki Y, Uchiyama K, Naito Y, Okanoue T, Yoshikawa T (2004) Lansoprazole ameliorates intestinal mucosal damage induced by ischemia-reperfusion in rats. World J Gastroenterol 10:2814–2817

Kobayashi T, Ohta Y, Inui K, Yoshino J, Nakazawa S (2002) Protective effect of omeprazole against acute gastric mucosal lesions induced by compound 48/80, a mast cell degranulator, in rats. Pharmacol Res 46:75–84

Konaka A, Kato S, Tanaka A, Kunikata T, Korolkiewicz R, Takeuchi K (1999) Roles of enterobacteria, nitric oxide and neutrophils in pathogenesis of indomethacin-induced small intestinal lesions in rats. Pharmacol Res 40:517–524

Kunikata T, Tanaka A, Miyazawa T, Kato S, Takeuchi K (2002) 16,16-Dimethyl prostaglandin E2 inhibits indomethacin-induced small intestinal lesions through EP3 and EP4 receptors. Dig Dis Sci 47:894–904

Kuroda M, Yoshida N, Ichikawa H, Takagi T, Okuda T, Naito Y, Okanoue T, Yoshikawa T (2006) Lansoprazole, a proton pump inhibitor, reduces the severity of indomethacin-induced rat enteritis. Int J Mol Med 17:89–93

Kwiecien S, Brzozowski T, Konturek SJ (2002) Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. J Physiol Pharmacol 53:39–50

Lai KC, Lam SK, Chu KM, Hui WM, Kwok KF, Wong BC, Hu HC, Wong WM, Chan OO, Chan CK (2003) Lansoprazole reduces ulcer relapse after eradication of Helicobacter pylori in nonsteroidal anti-inflammatory drug users: a randomized trial. Aliment Pharmacol Ther 18:829–836

Laine L (2001) Approaches to nonsteroidal anti-inflammatory drug use in the high risk patient. Gastroenterology 120:594–606

Laine L, Wogen J, Yu H (2003) Gastrointestinal health care resource utilization with chronic use of COX-2 specific inhibitors versus traditional NSAIDs. Gastroenterology 125:389–395

Lapenna D, de Gioia S, Ciofani G, Festi D, Cuccurullo F (1996) Antioxidant properties of omeprazole. FEBS Lett 382:189–192

Morini G, Grandi D, Arcari ML, Bertaccini G (1995) Gastroprotective activity of the novel proton pump inhibitor lansoprazole in the rat. Gen Pharmacol 26:1021–1025

Natale G, Lazzeri G, Lubrano V, Colucci R, Vassalle C, Fornai M, Blandizzi C, Del Tacca M (2004) Mechanisms of gastroprotection by lansoprazole pretreatment against experimentally induced injury in rats: role of mucosal oxidative damage and sulfhydryl compounds. Toxicol Appl Pharmacol 195:62–72

Ohara T, Arakawa T (1999) Lansoprazole decreases peripheral blood monocytes and intercellular adhesion molecole-1 positive mononuclear cells. Dig Dis Sci 44:1710–1715

Podolsky DK (2002) Inflammatory bowel disease. N Engl J Med 347:417–429

Reuter BK, Davies NM, Wallace JL (1997) Nonsteroidal anti-inflammatory drug enteropathy in rats: role of permeability, bacteria and enterohepatic circulation. Gastroenterology 112:109–117

Robert A, Asano T (1997) Resistance of germ-free rats to indomethacin-induced intestinal lesions. Prostaglandins 14:333–341

Robinson M (2004) Review article: the pharmacodynamics and pharmacokinetics of proton pump inhibitors: overview and clinical implications. Aliment Pharmacol Ther 20(Suppl 6):1–10

Satoh H, Inatomi N, Nagaya H, Inada I, Nohara A, Nakamura N, Maki Y (1989) Antisecretory and antiulcer activities of a novel proton pump inhibitor AG-1749 in dogs and rats. J Pharmacol Exp Ther 248:806–815

Seensalu R, Girma K, Romell B, Nilsson G (1990) Time course of inhibition of gastric acid secretion by omeprazole and ranitidine in gastric fistula rats. Eur J Pharmacol 180:145–152

Simon WA, Sturm E, Hartmann HJ, Weser U (2006) Hydroxyl radical scavenging reactivity of proton pump inhibitors. Biochem Pharmacol 71:1337–1341

Somasundaram S, Sightorsson G, Simpson RJ, Watts J, Jacob M, Tavares IA, Rafi S, Roseth A, Foster R, Price AB, Wrigglesworth JM, Bjarnason I (2000) Uncoupling of intestinal mitochondrial oxidative phosphorylation and inhibition of cyclooxygenase are required for the development of NSAID-enteropathy in the rat. Aliment Pharmacol Ther 14:639–650

Suzuki M (1995) Lansoprazole inhibits oxygen-derived free radical production from neutrophils activated by *Helicobacter pylori*. J Clin Gastroenterol 20(Suppl 2):S93–S96

Suzuki M, Mori M, Miura S, Suematsu M, Fukumura D, Kimura H, Ishii H (1996) Omeprazole attenuates oxygen-derived free radical production from human neutrophils. Free Rad Biol Med 21:727–731

Takahashi S, Yamazaki T, Okabe S (1997) Leminoprazole protects cultured gastric mucosal cells against damage caused by ethanol, indomethacin and taurocholate. Pharmacology 54:118–126

Thiéfin G, Beaugerie L (2005) Toxic effects of nonsteroidal antiinflammatory drugs on the small bowel, colon and rectum. Joint Bone Spine 72:286–294

Thomson RD, Lestina LS, Bensen SP, Toor A, Maheshwari Y, Ratcliffe NR (2002) Lansoprazole-associated microscopic colitis: a case series. Am J Gastroenterol 97:2908–2913

Tsuji S, Sun WH, Tsujii M, Kawai N, Kimura A, Kakiuchi Y, Yasumaru S, Komori M, Murata H, Sasaki Y, Kawano S, Hori M (2002) Lansoprazole induces mucosal protection through gastrin receptor-dependent up-regulation of cyclooxygenase-2 in rats. J Pharmacol Exp Ther 303:1301–1308

Uchiyama M, Mihara M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278

Wallace JL, Keenan CM, Granger DN (1990) Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. Am J Physiol 259:G462–G467

Wandall JH (1992) Effects of omeprazole on neutrophil chemotaxis, superoxide production, degranulation, and translocation of cytochrome b-245. Gut 33:617–621

Whittle BJR (2004) Mechanisms underlying intestinal injury induced by anti-inflammatory COX inhibitors. Eur J Pharmacol 500:427–439

Wilcox GM, Mattia A (2002) Collagenous colitis associated with lansoprazole. J Clin Gastroenterol 34:164–166

Wolfe MM, Lichtenstein DR, Singh G (1999) Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 340:1888–1899

Yamada T, Grisham MB (1991) Role of neutrophil-derived oxidants in the pathogenesis of intestinal inflammation. Klin Wochenschr 69:988–994

Yamada T, Deitch E, Specian RD, Perry MA, Sartor RB, Grisham MB (1993) Mechanisms of acute and chronic intestinal inflammation induced by indomethacin. Inflammation 17:641–662

Yeomans ND, Tulassay Z, Juhasz L, Racz I, Howard JM, van Rensburg CJ, Swannel AJ, Hawkey CJ (1998) A comparison of omeprazole with ranitidine for ulcers associated with nonsteroidal antiinflammatory drugs. Acid Suppression Trial: Ranitidine versus Omeprazole for NSAID-associated Ulcer Treatment (ASTRONAUT) Study Group. N Engl J Med 338:719–726

Yoshida N, Yoshikawa T, Tanaka Y, Fujita N, Kassai K, Naito Y, Kondo M (2000) A new mechanism for anti-inflammatory actions of proton pump inhibitors: inhibitory effects on neutrophil-endothelial cell interactions. Aliment Pharmacol Ther 14 (Suppl 1):74–81

Zedtwitz-Liebenstein K, Wenisch C, Patruta S, Parschalk B, Daxböck F, Graninger W (2002) Omeprazole treatment diminishes intra- and extracellular neutrophil reactive oxygen production and bactericidal activity. Crit Care Med 30:1118–1122