Proton Pump Inhibitors Exacerbate NSAID-Induced Small Intestinal Injury by Inducing Dysbiosis

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BACKGROUND & AIMS: Proton pump inhibitors (PPIs) and nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used classes of drugs, with the former frequently coprescribed to reduce gastroduodenal injury caused by the latter. However, suppression of gastric acid secretion by PPIs is unlikely to provide any protection against the damage caused by NSAIDs in the more distal small intestine. **METHODS:** Rats were treated with antisecretory doses of omeprazole or lanzoprazole for 9 days, with concomitant treatment with anti-inflammatory doses of naproxen or celecoxib on the final 4 days. Small intestinal damage was blindly scored, and changes in hematocrit were measured. Changes in small intestinal microflora were evaluated by denaturing gradient gel electrophoresis and reverse-transcription polymerase chain reaction. **RESULTS:** Both PPIs significantly exacerbated naproxen- and celecoxib-induced intestinal ulceration and bleeding in the rat. Omeprazole treatment did not result in mucosal injury or inflammation; however, there were marked shifts in numbers and types of enteric bacteria, including a significant reduction (~80%) of jejunal Actinobacteria and *Bifidobacteria* spp. Restoration of small intestinal Actinobacteria numbers through administration of selected (*Bifidobacteria* enriched) commensal bacteria during treatment with omeprazole and naproxen prevented intestinal ulceration/bleeding. Colonization of germ-free mice with jejunal bacteria from PPI-treated rats increased the severity of NSAID-induced intestinal injury, as compared with mice colonized with bacteria from vehicle-treated rats. **CONCLUSIONS: PPIs exacerbate NSAID-induced intestinal damage at least in part because of significant shifts in enteric microbial populations. Prevention or reversal of this dysbiosis may be a viable option for reducing the incidence and severity of NSAID enteropathy.**

Keywords: Ulcer; Bleeding; Acid Secretion; Microflora.

BASIC AND TRANSLATIONAL AT

The ability of nonsteroidal anti-inflammatory drugs (NSAIDs) to cause damage in the stomach is well-known, but these drugs also have the capacity to cause clinically significant injury in the small and large intestine. Approximately 70% of chronic NSAID users exhibit small intestinal inflammation,[1] which is associated with bleeding, strictures, and occasionally perforations.[2] The pathogenesis of NSAID enteropathy appears to be distinct from that of NSAID gastropathy.[3] Suppression of prostaglandin synthesis by NSAIDs renders the intestinal mucosa more susceptible to injury and less efficient in undergoing repair,[4,5] but, unlike the case for the stomach, a primary role of cyclooxygenase (COX) inhibition in the mechanism of NSAID-induced enteropathy is not clear.[4] On the other hand, the enterohepatic recirculation of NSAIDs and their secretion in bile are primary factors in the production of intestinal damage, coupled with their direct cytotoxic actions on enterocytes.[4,6,7] Enteric gram-negative bacteria also contribute significantly to NSAID-induced intestinal damage.[8] Germ-free mice do not develop intestinal ulcers when given NSAIDs,[9] but, when colonized by conventional bacteria, they become susceptible to NSAID-induced intestinal ulceration.[10] Broad-spectrum antibiotics have been shown to markedly reduce NSAID-induced small intestinal ulceration in animals.[11,12] Furthermore, mice that lack the receptor for bacterial endotoxin (Toll-like receptor 4) do not develop intestinal damage when given an NSAID.[13]

Proton pump inhibitors (PPIs) substantially reduce the incidence of NSAID-induced gastroduodenal damage.[14] On the other hand, acid does not appear to contribute significantly to NSAID-induced damage distal to the ligament of Treitz, and protective effects of PPIs against NSAID-induced small intestinal damage in humans have not been reported.[15] Recent video capsule endoscopy studies suggest a very high incidence of small intestinal damage in young, healthy, human subjects taking both an NSAID and a PPI for 2 weeks (55%–75% vs 7%–11% in placebo treated).[16–19] This suggests that the PPI conferred little, if any, protection to the mid- and distal small intestine, which are major sites of NSAID-induced bleeding.[1,20]

Gastric acid can kill most bacteria, and chronic suppression of acid can lead to bacterial overgrowth in the stomach and small intestine.[21–23] Given the apparent importance of gram-negative bacteria in the pathogenesis of NSAID enteropathy, it is possible that suppression of acid

Abbreviations used in this paper: CBS, cystathionine β-synthase; CFU, colony-forming units; COX, cyclooxygenase; CSE, cystathionine γ-lyase; DGGE, denaturing gradient gel electrophoresis; MRS, Man, Rogosa & Sharpe; NSAID, nonsteroidal anti-inflammatory drugs; PCR, polymerase chain reaction; PG, prostaglandin; PPI, proton pump inhibitor; mRNA, messenger RNA.

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secretion by a PPI could exacerbate NSAID-induced small intestinal damage. In the present study, we tested this hypothesis using an established animal model of NSAID-induced enteropathy and using doses of the test drugs that were effective in blocking their target enzymes.

Materials and Methods

Animals

Male Wistar rats weighing 180–220 g were obtained from Charles River (Montreal, QC, Canada) and were housed in the Central Animal Facility at McMaster University. The rats were fed standard chow and water ad libitum. Germ-free National Institutes of Health (Bethesda, MD) Swiss mice (male, 8 weeks of age) were raised in the Farncombe Institute Axenic Gnotobiotic Facility, as described previously.[24] All experimental procedures described herein were approved by the Animal Care Committee of the Faculty of Health Sciences at McMaster University, and the studies were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Suppression of Acid Secretion

To confirm that omeprazole and lansoprazole, at the selected dose (10 mg/kg twice daily), were significantly suppressing gastric acid secretion, rats treated for 5 days with these drugs or with vehicle were anesthetized with isoflurane, and the pylorus was ligated (the rats were deprived of food, but not water, for 18 hours prior to this procedure). The rats were allowed to recover from the anesthetic. Three hours later, the volume and titratable acidity of the luminal fluid were determined as described previously.[25]

NSAID-Induced Enteropathy

Following collection of a blood sample (75 μL) from the tail for determination of initial hematocrit, rats were treated twice daily with omeprazole, lansoprazole (both at 10 mg/kg intraperitoneally) or vehicle for a total of 9 days. In some experiments, naproxen (10 mg/kg) or vehicle was administered orally twice daily for the final 4 days of PPI/vehicle administration. The dose of naproxen was selected based on previous studies that demonstrated that it was effective in reducing inflammation in a rat adjuvant arthritis model,[26] that is suppressed systemic COX-1 activity by >95%, and that it suppressed gastric prostaglandin synthesis by >85%.[27] Moreover, on a per kilogram basis, the selected dose is similar to that most commonly used by humans with osteoarthritis (500 mg twice daily). Four hours after the final administration of drug or vehicle, hematocrit was measured, and the extent of hemorrhagic damage in the small intestine was blindly measured (the cumulative length, in millimeters, of all lesions). Additional studies were performed in which rats were treated with celecoxib (10 mg/kg) instead of naproxen.

Pharmacokinetics of Naproxen

The effect of omeprazole on plasma and biliary naproxen levels was determined as described in the legend of Supplementary Figure 1. Concentrations of naproxen in the bile and plasma samples were determined by high-performance liquid chromatography.[28]

Effects of Omeprazole on Intestinal Mucosal Integrity

Rats treated with omeprazole (10 mg/kg) or vehicle twice daily for 9 days then anesthetized with isoflurane. A blood sample was taken from the inferior vena cava for measurement of whole blood thromboxane synthesis, as an index of systemic COX-1 activity.[29] Formalin-fixed jejunal tissue was fixed for blind histologic examination (H&E staining). Additional jejunal tissue was snap frozen in liquid nitrogen for quantitative real-time polymerase chain reaction (PCR) analysis of messenger RNA (mRNA) expression for COX-1, COX-2, endothelial nitric oxide synthesis, tumor necrosis factor (TNF) α, cystathionine γ-lyase (CSE), and cystathionine β-synthase (CBS)[30] and for measurement of prostaglandin (PG)E_2 and hydrogen sulfide synthesis.[29,31] Blood samples were collected for measurement of serum levels of various cytokines and chemokines (Quansys Biosciences, Logan, UT).

Effects of a PPI on Enteric Microflora

Preliminary studies were focused on aerobic bacteria and are described in the legend of Supplementary Figure 1.[32] Subsequently, more extensive analysis of colonization of jejunum and colon by aerobic and anaerobic bacteria was performed for analysis of any marked changes in the microbiota after administration of omeprazole or vehicle for 9 days, as above. Samples of the jejunum and colon, with the luminal contents preserved, were flash frozen in liquid nitrogen. The tissue samples (and luminal contents) were further processed for denaturing gradient gel electrophoresis (DGGE), as described below.

Bacterial DNA/RNA was extracted from biologic samples as previously described.[33] The hypervariable v4 region of the bacterial 16S ribosomal DNA gene was amplified using PCR or reverse-transcription polymerase chain reaction (RT-PCR) with universal bacterial primers (HDA1-GC, HDA-2) (Mobixlab, McMaster University core facility, Hamilton, Canada) as previously described.[34] DGGE was carried out as previously described.[35,36] A scanned image of an electrophoretic gel was used to measure the staining intensity of the fragments using Quantity One software (version 4.2; Bio-Rad Laboratories, Hercules, CA). The intensity of fragments is expressed as a proportion (%) relative to the sum of the intensities of all of the fragments in the same lane of the image.[37]

Identification of bacterial phylogenies from DNA bands or bacterial colonies was performed as previously described.[34] PCR products were first checked by DGGE before being sent for sequencing using the didoexy method.[38] The retrieved sequences was compared with sequences among the Ribosomal Database Project (RDP)-II and National Center for Biotechnology Information GenBank (Bethesda, MD) databases using the maximum likelihood algorithm, and the sequences were used to represent phylotypes. In addition, real-time PCR was performed to determine the presence of Actinobacteria, *Bifidobacter* spp, and various specific *Bifidobacteria* species in jejunal samples from rats that had been treated with omeprazole or vehicle, as above (see Supplementary Tables 1 and 2).

Effects of Administration of Selected Commensal Bacteria on PPI-Induced Dysbiosis and NSAID Enteropathy

Commensal bacteria were isolated from samples of jejunal luminal contents from healthy rats by culture on Man, Rogosa & Sharpe (MRS) complemented with cysteine (0.5 g/L) and mupirocin (50 mg/L) and grown anaerobically at 37°C for