Suppression of contractile activity in the small intestine by indomethacin and omeprazole

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat a number of conditions and proton pump inhibitors (PPIs) are often used to prevent NSAID-induced gastric mucosal damage; however, the effects of NSAIDs on intestinal motility are poorly understood. The purpose of the present study is to determine the effects of a prototypical NSAID, indomethacin, either alone or in conjunction with the PPI, omeprazole, on intestinal motility. Rats were randomly divided into four groups treated with vehicle, omeprazole, indomethacin or a combination of indomethacin and omeprazole. Intestinal motility and transit were measured along with inflammatory mediators in the intestinal smooth muscle, markers of mucosal damage, and bacterial counts in the intestinal wall. Indomethacin, but not omeprazole, caused mucosal injury indicated by lower gut bleeding; however, both omeprazole and indomethacin suppressed contractile activity and frequency in the distal part of the small intestine. Co-treatment with omeprazole did not reduce indomethacin-induced intestinal bleeding. Furthermore, while indomethacin caused increased inflammation as indicated by increased edema development and inflammatory mediators, co-treatment with omeprazole did not reduce inflammation in the intestinal smooth muscle or prevent the increased bacterial count in the intestinal wall induced by indomethacin. We conclude that both NSAID and PPI treatment suppressed contractile activity in the distal regions of the small intestine. The suppression of intestinal contractility was associated with increased inflammation in both cases; however, indomethacin and omeprazole appear to affect intestinal motility by different mechanisms.

Keywords: indomethacin, omeprazole, intestinal motility, inflammation
Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of a number of different conditions including rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, and patent ductus arteriosus. The association of NSAID use with mucosal injury in the gastrointestinal tract is well established. NSAIDs have been shown to cause mucosal damage, not only in the stomach, but also in the small and large intestine (9, 17, 18, 21). In a recent study, lower GI damage accounted for 30-40% of all serious GI events in patients taking NSAIDs (17). However, side effects related to motility disorders are also common with NSAID use including nausea, vomiting, abdominal pain and constipation (13). The effects of NSAID use on gastrointestinal smooth muscle function are not well understood.

Several studies have shown that indomethacin induces increased motility in the lower gut (15, 24, 33). Nylander et al. showed that indomethacin treatment (3 mg/kg) increased motility in the duodenum of rats, measured as increased luminal pressure (24). Satoh showed that indomethacin (5 mg/kg) increased both the amplitude and frequency of contractions in the ileum when administered after feeding but not in fasted cats (33). Korolkiewicz et al. showed that indomethacin (30 mg/kg) attenuated the inhibitory effects of gut manipulation on intestinal transit (15). The Nylander, Satoh and Korolkeiwicz studies all show that indomethacin increases intestinal motility; however, in contrast to these studies, constipation has long been recognized as a common side effect of NSAIDs suggesting that NSAIDs reduce intestinal motility (13, 37, 38). Furthermore, NSAID use was found to be a risk factor for chronic constipation (6). Constipation would suggest a decrease in intestinal motility, not an increase as shown
in earlier studies and this discrepancy in experimental data versus known side effects of indomethacin warrants further study.

Proton pump inhibitors (PPIs) are often used in conjunction with NSAIDs to reduce the incidence of NSAID-induced gastric mucosal damage. Wallace et al. recently showed that PPIs can exacerbate NSAID-induced mucosal injury in the small intestine (44). However, the effects of PPIs on small intestinal motility are unknown. The effects of PPIs on small intestinal bacterial overgrowth (SIBO) have been somewhat controversial. Several studies reporting dysbiosis with PPI use (44, 46), including a meta-analysis; in contrast, other studies, including a large clinical study, showed no association of SIBO with PPI use (20, 29). Intestinal motility changes may influence whether SIBO develops in patients taking PPIs and NSAIDs. Thus, the effects of NSAIDs and PPIs on motility in the small intestine are important. The purpose of the present study is to determine the effects of a prototypical NSAID, indomethacin, either alone or in conjunction with the PPI, omeprazole, on intestinal motility.

**Materials and Methods**

*Animal Model.* All procedures were approved by the University of Texas Medical School Institutional Animal Care and Use Committee and are consistent with the NIH "Guide for the Care and Use of Laboratory Animals". Male Sprague Dawley rats weighing between 250-300 g were used for all experiments. Rats were randomly divided into 4 groups. The CONTROL group was vehicle treated only (saline). The PPI group was given Omeprazole (SQ, 37.5 mg/kg twice a day for 4 days). This dosage of omeprazole has been shown to suppress gastric acid secretion by approximately 90%
in rats (36, 44). The INDO group was given a single 20 mg/kg dose of indomethacin, subcutaneously, 24 hours before sacrifice (on third day of omeprazole treatment). The PPI/INDO group was given a combination of Omeprazole and Indomethacin as described above.

**Luminal Hemoglobin Content.** The intestinal luminal contents were collected by flushing 8 ml of cold 0.9% saline into the proximal duodenum and collecting the perfusate at the ileocecal valve. The flushed sample was kept at 4C, vortexed for 2 minutes, and centrifuged (3 min at 2000 rpm). Hemoglobin content was measured in the supernatants as described by Crosby and Furth (8). In a subset of rats in the indomethacin and control treatments only, the small intestine was divided into 10 equal sections, flushed separately, and the hemoglobin was measured in each intestinal segment.

**Measurement of Intestinal Contractility.** Intestinal contractile activity, in the longitudinal axis, was measured as described in (7, 25). Full thickness intestinal strips (approximately 10 mm in length) from ileal or jejunal sections of the small intestine, two from each section, were mounted in 25-ml organ baths filled with Krebs solution (in mM: 103 NaCl, 4.7 KCl, 2.5 CaCl₂, 25 NaHCO₃, 1.1 NaH₂PO₄, 15 glucose). The solution was buffered with albumin to avoid edema formation during incubation in the tissue chamber and gassed with 5% CO₂-95% O₂. Isometric force was monitored by an external force displacement transducer (Experimetria Ltd., Budapest, Hungary) connected to a PowerLab (AD Instruments, Colorado Springs, CO). Each strip was stretched to 0.5 g tension and allowed to equilibrate for 30 min. After equilibration, 10 min of basal contractile activity data were recorded. After recording contractile activity, length of each
strip was measured and tissue was removed, dried and weighed. Contractile activity parameters were all calculated over 5 minutes of recorded data. Total contractile activity was calculated as the area under the curve (Integral from minimum). Amplitude was calculated as average cycle height. All force development was normalized to tissue cross-sectional area. All measurements were performed in duplicate on two separate intestinal strips and averaged.

*Intestinal Transit.* A silastic catheter was introduced into the proximal duodenum of rats via vertical laparotomy incision for transit measurements. The catheter was tunneled through the musculature of the left abdominal wall and subcutaneous tissue and externalized behind the neck. The following day, a solution of 70 kD nonabsorbable fluorescein isothiocyanate (FITC) Dextran (150 μl) was injected into the duodenum via the catheter in awake animals. Forty-five minutes after administration of FITC Dextran animals were sacrificed. The entire small intestine was removed. The small intestine was divided into 10 equal segments and each segment was flushed with 3ml of 10% mM Tris-buffer solution (TBS). FITC-Dextran concentrations were measured in the flushed contents on a fluorescent plate reader (excitation 480, emission 520) in each sample and expressed as absorbance units (AU). Geometric center was calculated based on a modified method published by Miller et al. (23) using the following equation: geometric center = \sum(fraction of FITC per segment x segment number).

*Intestinal Interstitial Edema.* Wet to dry weight measurements were calculated as a measure of edema development. Full thickness intestinal samples were weighed immediately after collection. After drying in a 65°C oven, samples were weighed again. Wet to dry weight ratio was calculated as \(((\text{wet weight}) - (\text{dry weight})))/\text{dry weight}. 


**Nuclear Factor- kappa B (NF-kB) Assay.** NF-kB activation was measured in the nuclear extracts using a Transcription Factor Assay Kit (Active Motif, Carlsbad, CA) following manufacturer’s directions. Briefly, nuclear extracts from each group were added to each well of a 96 well plate in which oligonucleotide containing the NF-kB consensus sequence was immobilized. After incubation with lysates, a NF-kB p65 antibody was added followed by incubation with a secondary antibody conjugated to the horse radish peroxidase (HRP) enzyme. A colorimetric HRP substrate was then added. The colorimetric reaction was stopped with oxalic acid. Wells were washed after each incubation period. The plate was read at 450 nm wavelength. A recombinant P65 standard was used to generate a standard curve. Specificity of the assay was confirmed by competition with wild-type or mutant NF-kB consensus site oligonucleotides. Each sample was assayed in duplicate and normalized to total nuclear protein.

**Cytokine Array.** A rat cytokine array was utilized to measure cytokine and chemokine levels in the rat intestinal smooth muscle (R&D Systems, Minneapolis, MN) following manufacturer’s directions. In the cytokine array kit, capture antibodies for the following 29 cytokines and chemokines are immobilized on nitrocellulose membrane: Cytokine-induced neutrophil chemoattractant (CINC)-1, CINC-2α/β, CINC-3, ciliary neurotrophic factor (CNTF), fractalkine, granulocyte macrophage colony-stimulating factor (GM-CSF), soluble intercellular adhesion molecule (sICAM)-1, interferon (IFN)-γ, interleukin (IL)-1α, IL-1β, IL-1 receptor agonist (ra), IL-2, IL-3, IL-4, IL-6, IL-10, IL-13, IL-17, interferon gamma-induced protein (IP)-10, lipopolysaccharide-induced CXC chemokine (LIX), L-selectin, monokine induced by interferon-gamma (MIG),
macrophage inflammatory protein (MIP-1\(\alpha\)), MIP-3\(\alpha\), regulated upon activation normal T-cell expressed (RANTES), thymus chemokine, tissue inhibitor of metalloproteinase (TIMP)-1, tumor necrosis factor (TNF)-\(\alpha\), and vascular endothelial growth factor (VEGF).

Cytoplasmic extracts from smooth muscle obtained from the distal small intestine were prepared. Cytoplasmic extracts (300 \(\mu\)g) were diluted to the required volume and incubated with biotinylated detection antibodies for 1 hour at room temperature. After blocking the membrane, the sample/detection antibody mixture was incubated on the membrane overnight at 4°C. The membrane was then washed followed by incubation with streptavidin-horse radish peroxidase (HRP). After incubation with the chemiluminescent reagent, the membrane was exposed to Xray film and developed. Spots were quantitated using ImageJ (34).

**Bacterial Counts.** Full thickness small intestinal samples were collected in a sterile environment and flushed with sterile 0.9% saline. Intestinal tissue was chopped into 1 mm\(^3\) pieces, weighed, and placed in stomacher bags along with 3 ml sterile 0.9% saline for homogenization. Samples were blended for 2-3 minutes. After blending, serial dilutions were made up to \(10^{-7}\) and 25 ul of each dilution was plated on Brain Heart Infusion (BHI) agar for total aerobes. After incubation for 24 hours at 37°C, bacterial counts (total aerobes) were performed. Bacterial counts were divided by total weight of tissue blended.

**Statistics.** All data except for bacterial counts are shown as means ± standard error. Bacterial counts were logarithmically transformed and are shown as geometric means. Data was analyzed by one-way ANOVA.
Results

**Intestinal Injury.** The hematocrit decreased significantly in the INDO and PPI/INDO groups compared to the CONTROL group (Figure 1A) (INDO, 0.31 ± 0.017 and PPI/INDO, 0.27 ± 0.022 vs. CONTROL, 0.46 ± 0.0053) which is indicative of the development of anemia caused by NSAID-induced GI bleeding. PPI alone (0.45 ± 0.010) did not induce any significant change in hematocrit (PPI vs. CONTROL, p=0.59), and there were no significant differences in hematocrit between INDO and PPI/INDO groups (INDO vs. PPI/INDO, p=0.17).

As shown in Figure 1B, hemoglobin in the small intestinal lumen increased significantly in both the INDO and PPI/INDO groups compared to the vehicle treated CONTROL group (CONTROL, 18.08 ± 1.96 mg/dl; INDO, 696.50 ± 123.33 mg/dl; PPI/INDO, 688.74 ± 192.74 g/dl). Luminal hemoglobin was not altered compared to control values in the animals treated with Omeprazole only (PPI, 12.24 ± 1.93 g/dl) (CONTROL vs. PPI, p=0.97). Luminal hemoglobin after a combination of Omeprazole and indomethacin (PPI/INDO) treatment did not differ significantly from indomethacin treatment alone (INDO) (p=0.96). Panel C of Figure 1 shows luminal hemoglobin in the stomach and along the small intestine after indomethacin only treatment (INDO) or vehicle treatment. Hemoglobin increased significantly in the lumen of the distal part of the length of the small intestine only (intestinal sections 6-10), suggesting that indomethacin induced mucosal injury predominantly in the distal jejunum and ileum.

**Contractile Activity.** Contractile activity was measured in proximal and distal sections of the small intestine. As shown in Figure 2A, contractile activity was modestly
but significantly decreased in the jejunal region of the small intestinal after omeprazole treatment (CONTROL, 27.43 ± 2.70 g.s/cm$^2$ vs. PPI, 14.26 ± 2.74 g.s/cm$^2$) but not after indomethacin treatment or combination treatment with indomethacin and omeprazole (22.24 ± 3.31 and 19.02 ± 5.37, INDO and INDO/PPI, p= 0.32 and 0.13, respectively). Contractile activity in the distal (ileal) small intestine decreased significantly in all treatment groups compared to the CONTROL group (CONTROL, 49.20 ± 7.82 g.s/cm$^2$ vs. 15.54 ± 3.41, 32.14 ± 3.56, and 31.89 ± 2.91 g.s/cm$^2$ in PPI, INDO, and PPI/INDO groups, respectively) with changes in the PPI-treated group being most marked. In the jejunal region of the small intestine, the average contraction amplitude decreased significantly in the PPI group only compared to CONTROL (CONTROL, 0.12 ± 0.017 vs. PPI, 0.05 ± 0.008) as shown in Figure 2B. In the distal small intestine, the average contraction amplitude decreased significantly in all three treatment groups compared to CONTROL (CONTROL, 0.27 ± 0.044 vs. 0.10 ± 0.025, 0.16 ± 0.054, and 0.16 ± 0.039, PPI, INDO and PPI/INDO groups, respectively). Changes in contraction frequency in the jejunal and ileal sections of the small intestine are shown in Figure 2C. Frequency of contractions decreased significantly in the distal small intestine compared to the proximal small intestine as expected. Contraction frequency did not change significantly in the jejunum between the four treatment groups; however, frequency decreased significantly in all three treatment groups, PPI, INDO, and PPI/INDO, in the ileum compared to the CONTROL group (CONTROL, 0.40 ± 0.01 contractions/sec vs. 0.33 ± 0.005, 0.36 ± 0.01, and 0.29 ± 0.01 contractions/sec in PPI, INDO and PPI/INDO groups, respectively).
Intestinal transit was measured in awake, unanesthetized animals. Figure 3A shows the average % dye in each intestinal segment (proximal to distal, 1-10). The CONTROL and PPI groups exhibited a distinct dye peak corresponding to segments 6-7 in the mid-distal jejunum. In contrast, the INDO and PPI/INDO groups exhibited a broad, poorly defined dye peak. Quantification of the dye peak is shown in Figure 3B. The percent dye at the peak was significantly lower in the INDO and PPI/INDO groups compared to the CONTROL and PPI groups (CONTROL and PPI, 49.70 ± 3.97 and 65.76 ± 8.59 vs. INDO and PPI/INDO, 23.87 ± 1.59 and 29.84 ± 1.70). As shown in Figure 3C, transit was significantly slower (lower geometric center) in both the indomethacin treated group (INDO) and the indomethacin and omeprazole treated group (PPI/INDO) compared to the CONTROL group (CONTROL, 6.38 ± 0.43 vs. INDO, 4.9 ± 0.23 and PPI/INDO, 4.09 ± 0.42). Omeprazole treatment alone did not significantly alter intestinal transit (PPI, 6.36 ± 0.18, p=0.97 vs. CONTROL). Transit in the indomethacin treated group (INDO) was not significantly different from the INDO/PPI group (p=0.10).

**Inflammatory Markers.** Wet to dry weight ratios, indicating interstitial edema development, were measured in whole thickness distal small intestine in each group, as shown in Figure 4A. Wet to dry weight ratios increase significantly in all three drug treatment groups compared to CONTROL in the distal small intestine (3.44 ± 0.06 in CONTROL vs. 3.93 ± 0.07, 3.89 ± 0.10, and 3.98 ± 0.09 in PPI, INDO, and PPI/INDO groups, respectively). There were no significant differences in wet to dry weight ratios between the PPI, INDO, and PPI/INDO groups.
Nuclear NF-kB DNA binding activity was measured in distal intestinal smooth muscle as a marker of NF-kB activation as shown in Figure 4B. NF-kB activation increased significantly in the PPI and PPI/INDO groups but not in the INDO group compared to the CONTROL group (CONTROL, 8.36± 0.81 pg/mg nuclear protein vs. 15.65 ± 3.65, 10.84 ± 0.83, and 12.77 ± 1.06 pg/mg nuclear protein in PPI, INDO, and PPI/INDO groups, respectively).

Inflammatory cytokines were measured using an antibody-based microarray in distal intestinal smooth muscle. As shown in Figure 5A-C, inflammatory markers including IL-1α, IL-1β, and CINC-1 were significantly increased in the intestinal smooth muscle in the INDO and PPI/INDO groups compared to CONTROL. However, these inflammatory markers were not increased significantly in the PPI group. TIMP-1 and LIX-1 also increased significantly in both the INDO and PPI/INDO groups but not the PPI group (data not shown). MIP-1, and TNF-α increased significantly in the PPI/INDO group only but not the other groups (data not shown). On the other hand, IFN-γ increased significantly in the PPI group compared to CONTROL as shown in Figure 5D. IFN-γ did not increase significantly in the INDO or INDO/PPI groups (p=0.25 and 0.30 in INDO and INDO/PPI groups, respectively).

**Bacterial Growth.** Changes in total aerobic bacterial counts measured in whole thickness intestinal tissue were measured in CONTROL, PPI, INDO, and PPI/INDO groups as shown in Figure 6. Total aerobes increased significantly in the indomethacin treated groups (INDO and PPI/INDO) compared to both the CONTROL and the PPI group. PPI treatment had no significant effect on total aerobic bacterial growth (p=0.33, CONTROL vs. PPI). Treatment with both omeprazole and indomethacin (PPI/INDO)
not significantly increase bacterial growth in the small intestine compared treatment with indomethacin alone (INDO).

Discussion

Non-steroidal anti-inflammatory drugs (NSAIDS) including indomethacin are used extensively for inflammatory conditions such as rheumatoid arthritis and osteoarthritis. Both experimental and clinical studies have shown that NSAID use causes mucosal injury in both the stomach and small intestine (2, 4, 11). Proton pump inhibitors such as omeprazole are often used in conjunction with NSAIDs to prevent gastroduodenal mucosal injury (30, 31). However, the ability of PPIs to attenuate NSAID-induced lower gut injury has been questioned in several recent studies (44). In particular, recent studies show that PPIs protect against gastric mucosal damage but do little to protect the small intestine, a major site for NSAID-induced injury (19, 22). Although the effects of NSAIDs and PPIs on intestinal mucosa have been widely studied, the effects of these drugs on intestinal smooth muscle are unclear. We show, in the present study, that indomethacin, but not omeprazole, causes mucosal injury leading to lower gut bleeding (Figure 1); however, both omeprazole and indomethacin suppressed contractile activity and frequency in the distal part of the small intestine (Figure 2). Co-treatment with omeprazole did not reduce indomethacin-induced intestinal bleeding (Figure 1). Furthermore, co-treatment with omeprazole did not reduce inflammation in the intestinal smooth muscle or bacterial translocation induced by indomethacin (Figure 4 and 5). In addition to PPIs, Histamine H2 receptor antagonists, another class of anti-secretory drugs, have also been shown to exacerbate NSAID-induced injury to the duodenum;
however, the effects of these drugs on distal intestinal motility and injury should also be tested (5, 33).

The distribution of luminal hemoglobin (Figure 1C) suggests that mucosal injury/bleeding was predominantly limited to the distal part of the small intestine. While PPIs have been shown to protect the stomach from NSAID-induced damage (46), the protective effects of PPIs in the small intestine are unclear. While several studies have shown that PPIs can protect the small intestine from NSAID-induced mucosal injury, other studies have shown that PPIs exacerbate or fail to protect against NSAID-induced mucosal injury in the small intestine (19, 44). For example, lansoprazole was shown to prevent indomethacin-induced intestinal ulceration (46). On the other hand, Wallace et al. showed that omeprazole treatment significantly exacerbated NSAID-induced mucosal injury in the small intestine (44). We found no significant effect of PPI administration on indomethacin-induced small intestinal bleeding in our study. Differences in the studies may be a result of differences in the severity of injury due to treatment regime or type of NSAID used in the studies.

Spontaneous contractile activity in the proximal small intestine was unaffected by indomethacin treatment; however, omeprazole treatment inhibited contractile activity in the proximal small intestine alone or in combination with indomethacin (Figure 2A and C). Frequency of contractions were unaffected by either indomethacin or omeprazole treatment in the proximal part of the small intestine; however, the average contraction amplitude was suppressed by omeprazole treatment in the proximal small intestine (Figure 2B and C). The effects of PPIs on jejunal contractile activity may be related to PPI effects on gastric emptying (32). A large number of studies have shown that
omeprazole delays gastric emptying (3, 26-28, 40). We speculate that delayed gastric emptying may lead to decreased volume in the proximal small intestine and therefore, decreased contractile activity in the jejunum as shown in Figure 2.

Contractile activity in the ileal region of the small intestine was decreased in both the omeprazole and indomethacin treated groups. The combination of omeprazole and indomethacin did not significantly worsen contractile function compared to either treatment alone. Both frequency and average contraction amplitude were decreased in the treated groups compared to untreated and are likely responsible for the decreased contractile activity (Figure 2). Several investigators showed that indomethacin increased motility in the small intestine (24, 39). These studies were performed within 2-3 hours after administration of a lower dose of indomethacin. Furthermore, motility was measured via luminal pressure in the duodenum. Intestinal contractile activity in our study was measured 24 hours after administration of indomethacin and differences in contractility were detected predominantly in the ileum. Thus, discrepancies may reflect when and where contractile activity was measured. Furthermore, measurements in the organ bath system in our study may reflect smooth muscle dysfunction rather than neurally-mediated changes in motility. Kurt et al showed that omeprazole decreased spontaneous intestinal contractile activity when added to the organ bath (16). This study supports our data showing the inhibitory effects of omeprazole on intestinal smooth muscle contractility.

In agreement with the contractile activity, indomethacin treatment, either alone or in combination with omeprazole, slowed intestinal transit compared to the untreated group (Figure 3). The dye peak in the indomethacin treated groups was broad and the
percentage of dye at the peak in the INDO group was significantly lower compared to the Control group (Figure 3 A and B) indicating a less organized peristaltic contraction. These data along with the contractile data (Figure 2) suggest that both smooth muscle dysfunction and lack of coordination of peristaltic contractions occurred in the indomethacin treated groups, contributing to the slowed intestinal transit. Interestingly, although contractile activity, measured in the organ bath, decreased in the omeprazole treated group, intestinal transit was not significantly suppressed. Despite the decreased contraction amplitude, peristaltic contractions appeared to be well coordinated as demonstrated by the well-defined dye peak and the significantly higher percentage of dye at the peak in the omeprazole treatment group compared to the untreated group (Figure 3A and B). Intestinal transit was measured in fasted animals and duodenal pH has a significant effect on interdigestive migrating myoelectric complex (35, 45). Thus, the pH in the duodenum may have stimulated strong peristaltic contractions in the intact animals despite the dysfunctional smooth muscle. In the indomethacin treated groups, the dye peak was much more diffuse (Figure 3A) and the percent dye at the peak was significantly lower than in untreated control animals (Figure 3B).

Since most of the decreased contractile activity occurred in the distal part of the small intestine along with the mucosal injury as reflected by luminal hemoglobin, we measured inflammatory markers in the ileal smooth muscle. Intestinal edema developed after both omeprazole and indomethacin indicating the development of inflammation (Figure 4). These data indicate that smooth muscle dysfunction was likely due to inflammation in both omeprazole and indomethacin treatment; however, the type of inflammation appears to be different for omeprazole compared to indomethacin.
treatment as indicated by the differing pattern of inflammatory mediators (Figure 5). IFN-γ increased in the omeprazole-treated group, but not in the indomethacin treated groups. As no increases in luminal hemoglobin was detected in the omeprazole treatment group, the mechanism by which IFN-γ increased after omeprazole is unknown at this time and warrants further investigation. NF-kB activation was also increased in the intestinal smooth muscle after omeprazole treatment in the absence of any measurable mucosal damage (indicated by absence of hematocrit and luminal hemoglobin changes). IFN-γ is likely responsible for the increased NF-kB activation in the intestinal smooth muscle of the omeprazole treated group. NF-kB has been shown to suppress intestinal smooth muscle contractility by us and other investigators (10, 12, 43). For example, we showed that NF-kB activation inhibited intestinal contractile activity in a hydrostatic intestinal edema model (43). Increased NF-kB activation may be related to increased interferon-γ levels in the intestinal smooth muscle in the PPI group; however, the mechanism by which PPI increased interferon-γ is unclear. We did not detect increased bacterial growth in the omeprazole treated group, however, there may have been changes in a particular group of bacteria due to PPI-induced pH changes in the stomach and small intestine that could have caused the NF-kB activation and increased interferon-γ levels. Wallace et al demonstrated that omeprazole induced intestinal dysbiosis (44).

Contractile changes in the indomethacin treated groups (INDO and PPI/INDO) are likely due to mucosal damage leading to increased inflammation in the intestinal smooth muscle in this group. Inflammatory mediators including IL-1α, IL-1β and CINC-1 were significantly increased in the indomethacin treated groups due to indomethacin-
induced mucosal injury. Inflammation has been reported to both increase and decrease intestinal motility; thus, the effects of inflammation on motility are unclear and likely to depend on which inflammatory mediators are involved (1, 12, 14, 41, 42). IL-1β, in particular, is associated with suppression of both intestinal and colonic motility (12, 14, 41, 42). Murthy showed that IL-1β decreases colonic motility via down-regulation of CPI-17, an endogenous inhibitor of MLC phosphatase (12). Bauer showed that IL-1β increased and intestinal motility decreased in a peripheral traumatic injury model (42). Thus, increased IL-1β likely inhibits intestinal motility; however, the effects of IL-1α and CINC-1 on intestinal motility are less clear. In general, the effects of inflammatory mediators on smooth muscle function are poorly understood and warrant further investigations.

Bacterial counts measured in the intestinal wall from both indomethacin treated groups were significantly higher than the control group, indicating bacterial translocation in these groups (Figure 6). Both intestinal contractility and intestinal transit were compromised in these groups, our data suggest that the mucosal damage combined with decreased intestinal transit caused the increased bacterial translocation in these groups leading eventually to increased inflammation in the intestinal smooth muscle. The increased inflammation, in turn, likely contributed to the decreased intestinal motility and consequently the decreased intestinal transit through a feedback loop. Figure 7 shows the hypothetical model of intestinal smooth muscle injury induced by indomethacin.

One drawback of the study was that intestinal transit was measured in fasted animals, thus interdigestive contractile activity was measured. Decreased contractile...
activity in the organ bath occurred after both omeprazole and indomethacin treatment indicating intestinal smooth muscle dysfunction in both treatments. Further study is needed to determine if this smooth muscle dysfunction may have a more pronounced effect on intestinal transit of solid food compared to the liquid dye movement tracked in the present study.

In summary, both NSAID and PPI treatment suppressed contractile activity in the distal regions of the small intestine. The suppression of intestinal contractility was associated with increased inflammation in both cases; however, the mechanism appears to be different. Decreased contractility due to PPI appears to be due to increased IFN-γ and NF-kB activity while decreased motility after NSAID treatment is associated with increased IL-1β protein expression. In the long term, decreased intestinal motility may contribute to mucosal injury/bleeding and potentially bacterial overgrowth and translocation leading to increased septic complications. Our model of indomethacin-induced decrease in intestinal contractility is shown in Figure 7. Omeprazole decreased intestinal contractile activity, but did not appear to exacerbate and/or attenuate mucosal injury under our experimental conditions. The use of PPIs has been shown to have beneficial effects in the upper gastrointestinal tract; however, the protective effects of PPIs on small intestinal injury and transit in the fed state require further scrutiny.
Figure Legends

**Figure 1.** Hematocrits and luminal hemoglobin were measured as markers for mucosal injury in the small intestine. **Panel A.** Hematocrits measured on the day of sacrifice are shown for CONTROL, PPI, INDO, and PPI/INDO groups. (n=6 per group for CONTROL and PPI groups, n=7 for INDO and PPI/INDO groups, *, p<0.0001) **Panel B.** Total small intestinal lumen hemoglobin content is shown in CONTROL, PPI, INDO, and PPI/INDO groups. (mean ± SE are shown; n=12 per group for CONTROL and INDO groups; n= 6 for PPI and PPI/INDO groups; *, p<0.001) **Panel C.** Luminal hemoglobin content in CONTROL and INDO groups in the stomach (S) and along the length of the small intestine (SI-1 through SI-10) is shown. (mean ± SE are shown; n=4 per group; *, p<0.05)

**Figure 2.** Spontaneous contractile activity was measured in the proximal and distal small intestine in an isolated organ bath system. **Panel A.** Proximal and distal intestinal contractile activity, calculated as the integral from minimum is shown in CONTROL, PPI, INDO, and PPI/INDO groups. **Panel B.** The average contraction amplitude is shown in CONTROL, PPI, INDO, and PPI/INDO groups. **Panel C.** Frequencies of spontaneous contractions in the proximal and distal sections of the small intestine are shown in CONTROL, PPI, INDO, and PPI/INDO groups. (mean ± SE are shown; n=10 for CONTROL and INDO groups; n=6 for PPI and PPI/INDO groups; *, p<0.05; **, p<0.005)

**Figure 3.** Intestinal transit measured by propagation of non-absorbable FITC-Dextran dye through the small intestine is shown. **Panel A.** The average dye per intestinal segment is shown for each treatment group. **Panel B.** The percent dye at the peak
segment is shown for each treatment group. Panel C. The calculated geometric center reflecting the speed of intestinal transit is shown for each treatment group. (Mean ± SE are shown; n=6 per group; *, p<0.05; **, p<0.005)

Figure 4. Interstitial edema development and activation of NF-kB in the intestinal smooth muscle were measured as indicators of inflammation in the distal small intestine. Panel A. Wet to dry weight ratios, indicating edema development, in the distal small intestine in CONTROL, PPI, INDO, and PPI/INDO groups are shown. (mean ± SE are shown; n= 8 in CONTROL and INDO groups; n=4 in PPI and PPI/INDO groups; *, p<0.005) Panel B. Nuclear NF-kB DNA binding activity, indicating NF-kB activation, in smooth muscle of the distal small intestine from CONTROL, PPI, INDO, and PPI/INDO groups is shown. (mean ± SE are shown; n= 9 in CONTROL and INDO groups; n=5 in PPI and PPI/INDO groups; *, p<0.05)

Figure 5. An antibody-based inflammatory mediator array was used to measure changes in inflammatory mediators in the distal small intestinal smooth muscle. Changes in IL-1a (Panel A), IL-1b (Panel B), CINC-1 (Panel C), and IFN-g (Panel D) in intestinal smooth muscle from CONTROL, PPI, INDO, and PPI/INDO groups are shown. (mean ± SE are shown; n= 4, 5, 5, and 6 in CONTROL, PPI, INDO, and PPI/INDO groups, respectively; *, p<0.05; ** p<0.001)

Figure 6. The number of total aerobes in the full thickness small intestinal wall is shown for CONTROL, PPI, INDO, and PPI/INDO groups. (n=6-8 per group, mean is indicated for each group, ***, p<0.00001)
Figure 7. Model for indomethacin-induced inhibition of small intestinal smooth muscle contractility. Indomethacin causes mucosal damage which initiates a feedback loop involving inflammatory damage to smooth muscle layers which decreases intestinal smooth muscle contractility. The decreased intestinal contractility enhances the bacterial overgrowth and translocation initiated by the damaged mucosa.
References


A

Wet to Dry Weight Ratio

CONTROL  PPI  INDO  PPI/INDO

B

NF-kB Activity (pg/mg tissue)

CONTROL  PPI  INDO  PPI/INDO

* indicates significant difference

+ indicates trend towards significance
Total Aerobes Log_{10} CFU/gm

CONTROL PPI INDO PPI/INDO

p=0.33

***
Indomethacin

Mucosal Damage

Bacterial overgrowth and translocation

Decreased Intestinal contractility

Inflammation in smooth muscle layers