Intestinal motor disorders associated with cyclical bacterial overgrowth in a rat model of enteritis

Monica Porras, María Teresa Martín, Mercè Soler, and Patri Vergara

1Department of Cell Biology, Physiology and Immunology, and 2Centre de Recerca en Sanitat Animal, Universitat Autònoma de Barcelona 08193, Spain

Submitted 10 December 2003; accepted in final form 7 February 2004

Porras, Monica, María Teresa Martín, Mercè Soler, and Patri Vergara. Intestinal motor disorders associated with cyclical bacterial overgrowth in a rat model of enteritis. Am J Physiol Gastrointest Liver Physiol 287: G58–G64, 2004. First published February 12, 2004; 10.1152/ajpgi.00513.2003.—The aims of this study were: 1) to obtain an experimental model reproducing the characteristics of chronicity and spontaneous relapses found in inflammatory bowel disease (IBD) and 2) to correlate these changes with intestinal motility and bacterial translocation. For this purpose, two groups of Sprague-Dawley rats were used: a treated group that received two subcutaneous injections of indomethacin (7.5 mg/kg) 48 h apart and a control group that received saline. Blood leukocytes, TNF, and fecal parameters were monitored for 90 days after treatment. In treated rats, a cyclic oscillation of blood leukocytes and TNF concomitant with an inverse correlation of fecal output was observed. Treated rats were then selected either during their highest or lowest blood leukocyte values for motor activity and microbiological evaluation. Controls were obtained in age-matched rats. Rats with high leukocyte levels showed a decrease of motor activity. In contrast, animals with low leukocyte levels presented hypermotility. Bacterial overgrowth accompanied by bacterial translocation was found in the group with high leukocytes, whereas no differences were observed between the control and indomethacin groups during the lowest leukocyte phase. We obtained a model of IBD characterized by a chronic cyclic oscillation of intestinal motility, flora, and inflammatory blood parameters. During the high-leukocyte stage, motor activity decrease is related to bacterial translocation. This phase is followed by a reactive one characterized by hypermotility associated with a decrease in both bacterial growth and leukocytes. However, as in IBD, this reaction seems unable to prevent a return to relapse.

Inflammatory bowel disease; gastrointestinal motility; gut flora; leukocytes

Inflammatory Bowel Disease (IBD) in its more common forms, Crohn’s disease (CD) and ulcerative colitis, is characterized by presenting alternative phases of activity and quiescence and by its chronicity (11). Several studies (12, 22, 29) indicate an abnormal immune reactivity to intestinal flora as the cause for the inflammation, probably caused by impairment of the mucosal barrier function. Systemic administration of indomethacin in the rat has been used to cause inflammation of the small intestine, which presents some histopathological similarities to lesions found in CD (5, 37). It has been suggested that inhibition of cyclooxygenase activity induced by this drug alters mucosal permeability (7), facilitating the entry of bacteria and other harmful substances into the lamina propria and producing inflammation (24). Luminal bacteria seems to be critical to the induction of intestinal damage, because both germ-free rats (24) and animals treated with antibiotics (8) develop minimal lesions after the administration of indomethacin.

Although IBD patients present symptoms such as abdominal pain and diarrhea that suggest motor alterations, few motility studies have been performed (2). Moreover, it has been demonstrated that changes in motility are concomitant with inflammation, constituting a self-defense mechanism against the aggression (10, 34). Morphological and functional changes at the neuroendocrine structures controlling intestinal motility have also been observed using other models of inflammation such as those induced by nematode infection (30, 34).

Thus the objectives of our study were 1) to obtain a chronic model of enteritis using indomethacin, 2) to look for indicators of both active and reactive phases of the disease that can be easily determined, 3) to characterize small intestinal motility during both phases of the disease, 4) to evaluate the physiological mechanisms involved in the cyclical motor changes, and finally 5) to correlate motor changes with markers of inflammation, bacterial growth, and changes in bacteria location in the intestinal area.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River, Lyon, France), 8–10 wk old and weighing 300–350 g, were kept under conventional conditions in an environmentally controlled room (20–21°C, 12:12 h light-dark cycle) with water and standard laboratory rat chow available ad libitum. All experimental protocols were approved by the Ethical Committee of the Universitat Autònoma de Barcelona.

Experimental Model

Rats were randomized in two groups: 1) indomethacin group (n = 25) receiving two subcutaneous injections of indomethacin (7.5 mg/kg) 48 h apart and 2) control group (n = 12) receiving saline. Dosage of indomethacin was decided according to a preliminary validation study designed to indicate a dosage that would produce a moderate inflammatory reaction with absence of ascitis and a average body weight loss of not more than 10%. Body weight, food consumption, and number of fecal pellets were monitored daily. Blood samples were taken every 3 days between 10:00 and 11:00 AM to monitor both blood leukocytes (BL) by using a Neubauer chamber and TNF by a specific ELISA (rat TNF-α; Biosource International, Camarillo, California) with a minimum detectable concentration of 4 pg/ml. The B1 cyclic oscillation observed in indomethacin-treated rats enabled the selection of animals according to their BL level: 50% at high blood level (Hbl) an 50% at low blood level (Lbl). Studies were performed...
at different times, starting 15 days after and ≤90 days after treatment. An additional group of rats (n = 8) was studied just 2 days after indomethacin treatment to evaluate the lesions induced and the acute motility changes.

Motility Studies

Motor activity was evaluated in indomethacin-treated rats at acute phase (n = 4), Hbl (n = 8), and Lbl (n = 9) as well as in the control group (n = 8).

Animal preparation. At 12:00 AM, after a fasting period of 6 h, animals were anaesthetized by inhalation of halothane to allow cannulation of the right jugular vein as previously described (34). Level III of anesthesia was maintained with intravenous thiopental sodium as required. A laparatomy was performed to suture three strain gauges

Group (n) as required. A laparatomy was performed to suture three strain gauges

animalization of the right jugular vein as previously described (34). Level III of anesthesia was maintained with intravenous thiopental sodium as required. A laparatomy was performed to suture three strain gauges (3 × 5 mm; Hugo Sachs Elektronik) to the wall of the duodenum (at 2 cm from the pylorus), proximal jejunum (at 2 cm from Treitz’s ligament), and ileum (at 10 cm from cecum) to record circular muscle activity. Strain gauges were connected to high-gain amplifiers (MT8P; Lectromed), and signals were sent to a recording unit connected to a computer (PowerLab/800; ADInstruments). Finally, two electrode holders provided with two platinum electrodes each (World Precision Instruments, Sarasota, FL) were placed in the lumen 1 cm distally to the strain gauges of the duodenum and ileum, respectively, to induce ascending excitation of the peristaltic reflex by electrical mucosal stimulation (EMS) as previously described (14).

Evaluation of motor parameters. A protocol that has proven useful to investigate some mechanisms involved in the control of intestinal motor activity (32, 33, 34) was used. After an equilibration period of 10 min, spontaneous motor activity (SMA) was evaluated for 1 h. Afterward, CCK in octapeptide-sulfated form (CCK-8) (3 × 10⁻⁹ mol/kg) was intravenously infused during 10 min. After at least 1 h to allow the return to basal conditions, evaluation of both ascending inhibitory and excitatory innervation was performed by mucosal electrical stimulation of the duodenum and ileum at 30 V, 0.6 ms, and at 2 and 6 Hz (13). Each stimulus was applied for 30 s, and the polarity of the stimulating electrodes was reversed at 15 s. The interval between stimuli was 2 min. To evaluate the functionality of the nitrogenc pathways, N⁶-nitro-L-arginine (L-NNA) (10⁻⁵ mol/kg) was administrated intravenously, and the electrical stimulation protocol was repeated 15 min later. Finally, atropine (0.3 mg/kg) was intravenously infused as a bolus, and 5 min later the electrical stimuli were repeated to assess the contribution of both cholinergic and noncholinergic components in the excitatory motor response.

Microbiological Studies

Both luminal bacteria and intestinal wall bacteria were evaluated in indomethacin-treated animals [acute (n = 4), Hbl (n = 4), and Lbl (n = 4)] and in a control group (n = 4). Samples were obtained at different time intervals after treatment in a similar way to the motility studies. Rats were killed by CO₂ inhalation, and pieces of distal duodenum, jejunum, ileum, and colon were taken from the same animals used in microbiological studies. Tissues were fixed in 10% neutral buffered formalin and processed for histopathology according to standard procedures. Signs of inflammation were evaluated by hematoxylin and eosin staining.

Drugs and Solutions

Indomethacin (Sigma, St. Louis, MO) was dissolved in absolute ethanol (50 mg/ml) and diluted in a 0.1 M NaHCO₃ solution to obtain a 7.5 mg/kg dose in 0.3 ml final volume. CCK-8 (Peptide Institute, Osaka, Japan), was diluted in 1% NaHCO₃ solution to a concentration of 10⁻³ M and in buffered saline solution to work concentration. Atropine (Merck, Darmstadt, Germany) and L-NNA (Sigma) were diluted in saline solution.

Data Analysis

Data were expressed as means ± SE. Motor response was quantified measuring the area under the curve delimited by the tracing, and the results were expressed in square millimeters. Results of animal monitoring and of motility obtained in acute studies were analyzed using an unpaired t-test. Data obtained from motility studies at chronic stages were analyzed using ANOVA and Bonferroni’s post hoc test. In all cases, differences were considered significant when P < 0.05.

RESULTS

Animal Monitoring

Two days after treatment, the indomethacin group showed a decrease of food consumption (9.0 ± 1.9 vs. 29.4 ± 0.6 g in control group, P < 0.05) related to a reduced body weight gain (expressed as percentage of the initial weight) (−8.3 ± 1.2 vs. 5.9 ± 0.5%, P < 0.01). After the first week, these parameters returned to normal values. Moreover, a high increase of blood leukocyte levels was also observed, from 10,872 ± 140 to 25,695 ± 707 cells/mm³, followed by a cyclic oscillation of this parameter (Fig. 1A). This oscillation determined a high leukocyte (19,614 ± 222 cells/mm³; Hbl) and a low leukocyte (15,369 ± 259.7 cells/mm³; Lbl) phase. Despite observation of a steady decrease over the monitored 90 days, no animal was free of this cyclic oscillation. In contrast, BL concentration was quite constant in the control group (10,872 ± 140 cells/mm³; Fig. 1B), except for the normal decrease observed with aging (28). In a similar way, TNF in the same blood samples showed a parallel cyclical oscillation of its value in indomethacin rats, whereas TNF concentration was undetectable in control animals (Fig. 1, A and B).

Fecal pellets increased 24 h after the first dose of indomethacin (51.0 ± 0.8 pellets vs. 45.0 ± 1.2 pellets in control group, P < 0.05). Afterward, a cyclical oscillation in this parameter

using a sterile container with 3 ml sterile saline each time. Specimens were frozen in liquid nitrogen, powdered in a mortar kept at −80°C on dry ice, and weighted. Samples were homogenized in 9 ml milk (Difco, Detroit, MI) and incubated at appropriate dilution volumes following the same protocol as described above. Colony-forming units were also referred to a gram of intestinal tissue.

In both protocols, the mean concentration of isolated species found in each segment from control animals was considered to be the value of reference for that segment. Samples from indomethacin rats showing a result 10-fold higher than the control group were considered positive.

Histopathological Study

Samples of duodenum, jejunum, ileum, and colon were taken from the same animals used in microbiological studies. Tissues were fixed in 10% neutral buffered formalin and processed for histopathology according to standard procedures. Signs of inflammation were evaluated by hematoxylin and eosin staining.

Data Analysis

Data were expressed as means ± SE. Motor response was quantified measuring the area under the curve delimited by the tracing, and the results were expressed in square millimeters. Results of animal monitoring and of motility obtained in acute studies were analyzed using an unpaired t-test. Data obtained from motility studies at chronic stages were analyzed using ANOVA and Bonferroni's post hoc test. In all cases, differences were considered significant when P < 0.05.

RESULTS
was also observed, showing an inverse correlation with leukocyte and TNF values (Fig. 1C).

**Motility Studies**

Chronic-phase rats were studied from 15 to 90 days after treatment, and animals were selected at Hbl or Lbl phases. Acute-phase rats were studied at 2 days after indomethacin treatment.

**Chronic phase. SMA.** In the control group, SMA was characterized by isolated phasic contractions occurring at regular frequency. Treated rats at the Lbl phase presented an increase of SMA, with higher frequency of contractions and the appearance of clustered phasic contractions, whereas SMA during the Hbl phase was practically nonexistent (Fig. 2).

**RESPONSE TO CCK AND L-NNA.** In control rats, CCK caused an increase in motor activity in the duodenum (from 18.6 ± 7.3 to 602.8 ± 120.2 mm², P < 0.001). In the indomethacin group, CCK response was higher at the Lbl phase and lower during the Hbl phase, compared with the response observed in control animals (Fig. 3). CCK has an inhibitory effect in the jejunal similar to that observed in previous studies (13, 14, 34). This inhibitory response was not modified in indomethacin rats.

The increase of motor activity induced by L-NNA in control rats showed a tendency to be greatest at the Lbl phase of indomethacin-treated animals. However, the differences only reached statistical significance in the jejunal (242.4 ± 41.7 vs. 117.0 ± 23.4 mm² in control group, P < 0.05). By contrast, no differences were observed in the response to L-NNA between the Hbl and the control group.

**RESPONSE TO EMS.** In control rats, EMS at 2 and 6 Hz always induced an ascending contraction recorded at the oral strain gauge, both in the duodenum and ileum. This response was

![Fig. 1. A: representative graph showing blood leukocytes (BL) and TNF evolution after indomethacin treatment in an individual animal. B: representative graph showing BL evolution in a control animal. TNF was not detectable in this and other control animals. C: representative graph showing the oscillatory inverse correlation between BL and fecal pellets (FP) in an individual rat after indomethacin treatment.](image)

![Fig. 2. A: representative recordings of mechanical activity showing the spontaneous motor events in the duodenum of one animal for each experimental group. Similar recordings were found in all the animals of the same group. B: average of frequency of contraction observed in the duodenal spontaneous motor activity in each group. **Significant difference (P < 0.01) vs. control group; ++ significant difference (P < 0.01) between high blood level (Hbl) and low blood level (Lbl) groups.](image)
Increased by L-NNA and reduced by atropine. Differences in the response to EMS were observed according to the blood leukocyte stage of the animals.

In the Hbl group, a decrease in the magnitude of the response to EMS was observed in both studied segments at both frequencies of stimuli (Fig. 4). However, after L-NNA administration, the response to EMS was similar to the response after L-NNA in the duodenum of the control group. The ileal response after L-NNA was still lower than in the control group. In all cases, ascending contraction elicited after atropine administration was similar to the control group.

In the Lbl group, the response induced by stimulation at 2 Hz was not modified, whereas the contraction obtained after stimulation at 6 Hz was higher compared with the control group, especially in the ileum (Fig. 4). The response to EMS after L-NNA administration significantly increased in this group compared with the control group, and the magnitude of the remaining response after atropine in the ileum was also significantly higher.

**Acute phase.** The intestine of the indomethacin group did not show any SMA 2 days after the treatment. In contrast, no changes were observed in the CCK response in relation to the control group (490.9 ± 79.2 mm²), whereas a significant increase of activity was found after L-NNA infusion, both in the jejunum (343.4 ± 47.2 vs. 131.3 ± 35.4 mm² in control, P < 0.01) and ileum (323.0 ± 33.4 vs. 65.0 ± 31.3 mm² in control, P < 0.01). Response to EMS after L-NNA administration also significantly increased both in duodenum (2 Hz: 24.7 ± 5.1 vs. 7.8 ± 2.5 mm² in control, P < 0.01; 6 Hz: 56.0 ± 11.1 vs. 30.3 ± 10.0 mm² in control, P < 0.05) and ileum (2 Hz: 22.7 ± 6.7 vs. 3.0 ± 1.7 mm² in control, P < 0.01; 6 Hz: 48.8 ± 7.1 vs. 30.1 ± 6.8 mm² in control, P < 0.05), whereas no differences were observed after atropine administration in relation to the control group.

**HISTOLOGICAL STUDY.** Indomethacin induced an important acute inflammatory reaction in the small intestine, especially in the distal jejunum and proximal ileum. No changes were observed in the colon.

Macroscopically, alterations were observed until 15 days after treatment, characterized by a severe distention of the small bowel loops with an edematous appearance. White nodules were located on the serosal side of the mid-small intestine and multiple adherences were also found. Microscopically, damage was maximal at 4 days after treatment, showing an inflammatory infiltrate with neutrophil and macrophage cells, accompanied by an extensive edema that affected both the mucosa and serosa. Multifocal subepithelial vacuolization and multiple mucosal ulcers on the mesenteric side of the small intestine were also found.

In contrast, 15 days after treatment no evident microscopic differences were observed between control and treated rats at Hbl or Lbl phases using the conventional staining procedure previously described.

**MICROBIOLOGICAL STUDIES.** Despite the normal appearance of the intestine from 15 days after treatment, marked changes in both bacterial luminal load (Table 1) and enteric bacterial wall colonization (Table 2) were observed in the small intestine as well as in the colon.

Species isolated under anaerobic conditions were mainly facultative anaerobes (gram-positive flora) both in small intestine and colon. In addition, a few strict anaerobes were also identified in the colon (*Clostridium*). Isolated gram-positive flora identified include the following: *Enterococcus faecalis*, *Staphylococcus*, and *Lactobacillus*. The main enterobacteria cultured was *Escherichia coli*. Similarity of results from anaerobic and aerobic cultures allowed us to discard the anaerobic cultures and focus only on aerobic cultures.

In the acute phase, luminal enterobacteria increased in duodenum, jejunum, ileum, and colon, whereas a significant increase of gram-positive flora was only observed in colon. Moreover, bacterial translocation of both enterobacteria and gram-positive flora was found in all intestinal segments studied.

In the chronic phase, results obtained during the Lbl phase were similar to those observed in the control group. During the Hbl phase, an increase in the luminal enterobacteria was observed 30 days after treatment. Moreover, intestinal wall colonization of both enterobacteria and gram-positive flora was also detected in all segments studied.

**Fig. 3.** A: representative recordings of mechanical activity showing motor activity in duodenum in response to CCK-8 infusion (3 × 10⁻⁶ mol/kg) in one animal of each experimental group. Arrows indicate beginning and end of infusion. Similar recordings were found in all the animals of the same group. B: quantitative analysis of the response to CCK in the duodenum in each group. *Significant difference P < 0.05 vs. control group (unpaired r-test).**
DISCUSSION

This study demonstrates that there is a high correlation between intestinal motor activity, bacterial translocation, and indicators of inflammation. We reproduced in an experimental model the chronic relapses reported in IBD patients. In this model, alternative phases of motor activity and quiescence occurred. These phases could be predicted by blood leukocyte monitoring, as well as by the cyclic oscillation of TNF in serum. Hypomotility was related to high blood leukocyte and TNF concentration, whereas hypermotility was observed when leukocyte counting decreased to normal levels. Our results also show that high leukocyte values and hypomotility coincided with bacterial invasion of the intestinal wall.

Histological similarities between the rat indomethacin model and CD are well known (3, 5). However, because the described protocols using indomethacin cause extensive intestinal damage (19, 37), studies could only be performed a few

Table 1. Results from cultures of luminal specimens 2 days (acute) and 30 days (Hbl and Lbl) after indomethacin treatment

<table>
<thead>
<tr>
<th>Intestinal Area</th>
<th>MC</th>
<th>Acute</th>
<th>Hbl</th>
<th>Lbl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1: Gram + Flora*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>10⁷</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Jejunum</td>
<td>10⁷</td>
<td>0/4</td>
<td>4/4 (10⁸)</td>
<td>0/4</td>
</tr>
<tr>
<td>Ileum</td>
<td>10⁸</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Colon</td>
<td>10⁸</td>
<td>3/4 (10⁹)</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2: Enterobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
</tr>
<tr>
<td>Jejunum</td>
</tr>
<tr>
<td>Ileum</td>
</tr>
<tr>
<td>Colon</td>
</tr>
</tbody>
</table>

Results from luminal cultures in both acute and chronic studies are expressed as positive cases/total number of rats. Positive cases are samples showing bacterial concentrations 10-fold higher than the control group; MC, mean concentration of control group; Hbl, high blood level; Lbl, low blood level. *Includes facultative anaerobes.

Table 2. Results from cultures of intestinal wall specimens 2 days (acute) and 30 days (Hbl and Lbl) after indomethacin treatment

<table>
<thead>
<tr>
<th>Intestinal Area</th>
<th>MC</th>
<th>Acute</th>
<th>Hbl</th>
<th>Lbl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1: Gram + Flora*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>10⁰</td>
<td>4/4 (10⁴)</td>
<td>4/4 (10³)</td>
<td>0/4</td>
</tr>
<tr>
<td>Jejunum</td>
<td>10⁰</td>
<td>2/4 (10⁴)</td>
<td>2/4 (10⁴)</td>
<td>0/4</td>
</tr>
<tr>
<td>Ileum</td>
<td>10⁴</td>
<td>4/4 (10⁴⁰)</td>
<td>2/4 (10⁴)</td>
<td>0/4</td>
</tr>
<tr>
<td>Colon</td>
<td>10⁴</td>
<td>4/4 (10⁴⁰)</td>
<td>4/4 (10⁴)</td>
<td>0/4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2: Enterobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
</tr>
<tr>
<td>Jejunum</td>
</tr>
<tr>
<td>Ileum</td>
</tr>
<tr>
<td>Colon</td>
</tr>
</tbody>
</table>

Results from luminal cultures both in acute and chronic studies are expressed as positive cases/total number of rats. *Includes facultative anaerobes.
days after treatment. By modifying a previously reported protocol (27), we obtained rats with less-severe lesions, which allowed us to perform chronic studies. As previously reported (37), an initial inflammatory reaction was observed especially in the mid-small intestine, which was concomitant with enterobacteria overgrowth and wall invasion by both enterobacteria and gram-positive bacteria. Interestingly, a marked increase in wall invasion by bacteria was also observed in the colon despite the absence of any sign of inflammation, suggesting that functional alteration might also happen in noninflamed areas. As previously described (31), indomethacin markedly alters the return to basal values of both leukocytes and TNF values. Unfortunately, our results do not explain why, after a period of relative normality, the intestine returns to the active inflammatory phase. A prolonged period of intestinal permeability alteration has been demonstrated after inflammation (4). In consequence, our hypothesis is that bacterial translocation induces inflammatory reaction and thus hypermotility. Once hypermotility is established, both bacterial translocation and inflammatory reaction decrease, reducing the drive for hypermotility. However, if permeability remains altered, the decrease of motility could then favor a new bacterial translocation and the perpetuation of the disease. The high correlation between motility and active and reactive phases opens a new perspective on the study of the mechanisms implicated in IBD and its treatment.

In conclusion, we have obtained a chronic model of enteritis characterized by spontaneous cyclical alternation of active and reactive phases that are correlated with cyclical long-lasting motor changes. During the active phase, there is a decrease in motor activity that coincides with wall invasion by intestinal flora and an increase in the blood level of inflammatory indicators. This phase is followed by a reactive phase characterized by hypermotility that helps to restore both flora and BL values. However, as in IBD, this reaction seems unable to prevent a return to cyclic relapse.

ACKNOWLEDGMENTS

The authors thank A. Marco for the histological study, A. Acosta for care of the animals, and A. C. Hudson for editorial revision of the manuscript.

REFERENCES