Effect of a selective chloride channel activator, lubiprostone, on gastrointestinal transit, gastric sensory, and motor functions in healthy volunteers

Michael Camilleri,1 Adil E. Bharucha,1 Ryuji Ueno,3 Duane Burton,1 George M. Thomforde,1 Kari Baxter,1 Sanna McKinzie,1 and Alan R. Zinsmeister2

1Clinical Enteric Neuroscience Translational and Epidemiological Research Group and 2Division of Biostatistics, Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, Minnesota; and 3Sucampo Pharmaceuticals Incorporated, Bethesda, Maryland

Submitted 8 June 2005; accepted in final form 19 December 2005

Camilleri, Michael, Adil E. Bharucha, Ryuji Ueno, Duane Burton, George M. Thomforde, Kari Baxter, Sanna McKinzie, and Alan R. Zinsmeister. Effect of a selective chloride channel activator, lubiprostone, on gastrointestinal transit, gastric sensory, and motor functions in healthy volunteers. Am J Physiol Gastrointest Liver Physiol 290: G942–G947, 2006; doi:10.1152/ajpgi.00264.2005.—Chloride channels modulate gastrointestinal neuromuscular functions in vitro. Lubiprostone, a selective type 2 chloride channel (ClC-2) activator, induces intestinal secretion and has been shown to relieve constipation in clinical trials; however, the effects of lubiprostone on gastric function and whole gut transit in humans are unclear. Our aim was to compare the effects of the selective ClC-2 activator lubiprostone on maximum tolerated volume (MTV) of a meal, postprandial symptoms, gastric volumes, and gastrointestinal and colonic transit in humans. We performed a randomized, parallel-group, double-blind, placebo-controlled study evaluating the effects of lubiprostone (24 μg bid) in 30 healthy volunteers. Validated methods were used: scintigraphic gastrointestinal and colonic transit, SPECT to measure gastric volumes, and the nutrient drink (“satiation”) test to measure MTV and postprandial symptoms. Lubiprostone accelerated small bowel and colonic transit, increased fasting gastric volume, and retarded gastric emptying. MTV values were reduced compared with placebo; however, the MTV was within the normal range for healthy adults in 13 of 14 participants, and there was no significant change compared with baseline measurements. Lubiprostone had no significant effect on postprandial gastric volume or aggregate symptoms but did decrease fullness 30 min after the fully satiating meal. Thus the ClC-2 activator lubiprostone accelerates small intestinal and colonic transit, which confers potential in the treatment of constipation.

colon; secretion; motility; ion channel

NEURAL CONTROL of gut motor and sensory function is influenced by the action of transmitters and changes in ion channel function. Among these, anion channels appear to be important given the documented effects on membrane function (13).

The cystic fibrosis (CF) transmembrane conductance regulator (CFTR) is a CAMP-dependent Cl− channel located in the intestinal epithelium that is activated by protein kinase A-mediated phosphorylation. A major portion of apical Cl− transport occurs via CFTR (2). More recently, however, intestinal and colonic epithelial cells were also found to contain the type 2 Cl− channel (ClC-2), a member of the Cl− channel family, which consists of nine members that are widely distributed in nature (28).

Volume-regulated channels such as ClC-2 and ClC-3 are found in most mammalian and nonmammalian cell types including those in the gastrointestinal tract and liver (5, 11, 15, 23, 28–30, 33). These channels contribute to cell volume regulation, maintaining the membrane potential, intracellular pH regulation, epithelial Cl− transport and fluid secretion, exocytosis, transmembrane transport of organic osmolytes, and cell proliferation. In the gastrointestinal tract, ClC-2 has been found in gastric parietal cells and small intestinal and colonic epithelia. Specifically, immunofocal microscopy has shown ClC-2 to be localized in the apical membrane in human gastrointestinal epithelial cells (17). A recent study (17) demonstrated that ClC-2, but not CFTR, Cl− currents in a transfected human cell line were selectively activated by lubiprostone, a novel bicyclic fatty acid compound.

Lubiprostone (which also appears in the literature as SPI-0211 or RU-0211) is a member of a new class of bicyclic fatty acid compounds called prostones, which are derived from a metabolite of prostaglandin E1. However, unlike prostaglandins, prostones have little or no effect on prostaglandin E (EP) or F (FP) receptors and do not stimulate smooth muscle contraction (37). Lubiprostone has highly selective activity on ClC-2 (17). Activation of ClC-2 located in the gastrointestinal tract increases Cl− transport in the lumen and enhances intestinal fluid secretion (36).

Clinical studies (25–27) have demonstrated that lubiprostone is safe and effective for treating patients with chronic constipation. However, it is unknown whether activation of ClC-2 in humans accelerates small intestinal and/or colonic transit, particularly because the proximal colon can compensate by increasing absorption of fluid secreted into the small intestine. Therefore, our aims were to assess the effects of the novel ClC-2 activator lubiprostone on gastrointestinal and colonic transit as well as gastric motor and sensory functions in healthy male and female volunteers.

MATERIALS AND METHODS

Study Design, Participants, and Medication

This was a randomized, parallel-group, single-dose, multiple-administration, double-blind, placebo-controlled study evaluating the effects of orally administered 48 μg lubiprostone (24 μg twice daily...
Each dose consisted of one capsule (placebo or 24 µg lubiprostone, which were identical in appearance). Briefly, 111In absorbed on activated charcoal particles was delivered by oral ingestion, abdominal images were obtained every 15 min for the first 16 min in duration, and three were obtained: one during fasting and two other images sequentially after oral ingestion of 300 ml (1 kcal) of the liquid caloric drink Ensure (Ross Laboratories; Abbott Park, IL). The 99mTc SPECT technique involves the infusion of 99mTc, which is taken up by the gastric mucosa. Dynamic tomographic images of the gastric wall are obtained throughout the long axis of the stomach using a dual-head gamma camera (SMV SPECT System, SMV America; Twinsburg, OH) that rotates around the body. This allows the assessment of the radiolabeled gastric wall rather than the intragastric content. With the use of the AVW 3.0 (Biomedical Imaging Resource, Mayo Foundation; Rochester, MN) image-processing libraries, a three-dimensional rendering of the stomach was obtained, and its volume (in ml) was calculated.

Satiation and Postchallenge Symptoms

On two occasions, once after eligibility was confirmed before the randomized treatment was started and once after the treatment as described above, participants underwent the “satiation test” to assess the MTV and postchallenge symptoms (34). Subjects ingested 30 ml Ensure/min. The cup containing the nutrient drink was filled using a 1/2 of ascending colon emptying was also estimated by plotting the activity-time curve for percent residing in the ascending colon; linear interpolation was used to connect points.

G943

Transit data analysis. A variable region of interest program was used to quantitate the counts in the stomach and each of four colonic regions: ascending, transverse, descending, and combined sigmoid and rectum. These counts were corrected for isotope decay, tissue attenuation, and downscatter of 111In counts in the 99mTc window (6, 7, 9, 10, 16, 38).

The primary summaries obtained for comparison of transit profiles were gastric half-emptying time (t1/2), small bowel transit time (t10%; time for 10% colonic filling minus the gastric lag time), and colonic geometric center at 24 h. The geometric center is the weighted average of counts in the different colonic regions: ascending (AC), transverse (TC), descending (DC), rectosigmoid (RS), and stool. At any time, the portion of colonic counts in each colonic region is multiplied by its weighting factors as follows:

\[
\frac{\%AC \times 1 + \%TC \times 2 + \%DC \times 3 + \%RS \times 4 + \%stool}{5} = \text{geometric center}
\]

Thus a high geometric center implies faster colonic transit. A geometric center of 1 implies that all isotope is in the ascending colon, and a geometric center of 5 implies that all isotope is in the stool. The t1/2 of ascending colon emptying was also estimated by plotting the activity-time curve for percent residing in the ascending colon; linear interpolation was used to connect points.

99mTc SPECT to Measure Gastric Volume

The accommodation response to meal ingestion is a robust, vagally mediated reflex in health. It results in a reduction in gastric tone and an increase in compliance, thereby facilitating the ingestion of large volumes of solids or liquids without inducing postprandial symptoms such as pain, bloating, or the vomiting reflex. On one study day, subjects underwent SPECT imaging to measure fasting and postprandial gastric volumes. The SPECT technique involves the infusion of 99mTc, which is taken up by the gastric mucosa. Dynamic tomographic images are obtained with the SPECT camera, allowing visualization of three-dimensional images of the stomach. The images are 16 min in duration, and three were obtained: one during fasting and the two other images sequentially after oral ingestion of 300 ml (1 ml = 1 kcal) of the liquid caloric drink Ensure (Ross Laboratories; Abbott Park, IL). The 99mTc SPECT technique accurately and reliably demonstrates changes in gastric volume. A previous validation study (3) compared volume responses measured simultaneously by the SPECT method and by a barostatically controlled intragastric balloon in response to nutrient meal ingestion or to standard balloon distension. In this method, tomographic images of the gastric wall are obtained throughout the long axis of the stomach using a dual-head gamma camera (SMV SPECT System, SMV America; Twinsburg, OH) that rotates around the body. This allows the assessment of the radiolabeled gastric wall rather than the intragastric content. With the use of the AVW 3.0 (Biomedical Imaging Resource, Mayo Foundation; Rochester, MN) image-processing libraries, a three-dimensional rendering of the stomach was obtained, and its volume (in ml) was calculated.

Gastric volume data analysis. Fasting and postprandial gastric volumes were measured by ANALYZE using reconstructed, three-dimensional images of the stomach (3). Two time periods, 0–16 and 17–32 min after the meal, were assessed, and the average of these two postprandial gastric volume estimates was calculated and used in the analysis of postprandial volumes.
constant rate perfusion pump, and participants were required to maintain intake at the filling rate until the MTV was reached. Participants scored their satiation (feeling of fullness) at 5-min intervals using a graphic rating scale that combined verbal descriptors on a scale graded 0–5 (where 0 = no symptoms and 5 = maximum satiation).

Thirty minutes after reaching the MTV, participants scored their symptoms of bloating, fullness, nausea, and pain using 100-mm visual analog scales (VAS) anchored with the words “unnoticeable” and “unbearable” at the left and right ends (i.e., maximum score of 100 for each symptom). The aggregate symptom score was defined as the sum of the VAS scores for each symptom (i.e., maximum score of 400). We (14) have previously reported normal values for the nutrient drink test in adolescents and adults.

Satiation and symptom data analysis. The MTV of nutrient drink ingested was recorded. Individual symptom scores (maximum score of 100) for nausea, bloating, fullness, and pain and the aggregate symptom score (maximum score of 400) were documented and analyzed.

Compliance with Study Medication

During the treatment period, patients recorded their dosing with the study medication in their daily diary. Reasons for noncompliance were also recorded. Compliance was evaluated from the records in the patient’s diary and by the coordinator count of the number of unused capsules returned at the exit visit. Participants were considered compliant if they had not missed more than three doses of the study medication. However, immediately before the postmedication tests, all participants were documented to have received dosing in accordance with the study protocol.

Statistical Analysis

The primary response end points were gastric emptying \( t_{1/2} \), small bowel transit \( t_{1/2} \), geometric center at 24 h, ascending colon emptying \( t_{1/2} \), fasting and postprandial gastric volumes, and MTV. Comparisons of the active treatment group versus the placebo group were tested using analysis of covariance with the inclusion of gender, body mass index (BMI), and, for the satiation end points, the corresponding baseline tests as covariates. Significance was set at an \( \alpha \)-level of 0.05 for comparison of the main effect of drug versus the placebo.

Sample Size Assessment

Table 1 summarizes data for the primary response measures and uses the (relative) variation to estimate the effect size detectable with 80% power based on a two-sample \( t \)-test (i.e., assuming the variation values are known) at a two-sided \( \alpha \)-level of 0.05. The effect size is the difference in group means as a percentage of the overall mean for each response and assumes 15 subjects/group.

Data used for all end points are based on data acquired in our laboratory using the same methods proposed for this study and published in the literature (3, 6, 8–10, 14, 16).

RESULTS

Participants, Study Conduct, and Completion

Demographic and baseline data characterizing the responses in the satiation test are shown in Table 2. Two of the thirty-two subjects randomized did not complete the study; one volunteer who was randomized to receive lubiprostone did not take any medication, and one volunteer from the placebo group took only one dose before withdrawal. These two subjects were not included in the efficacy analyses, which are based on the remaining total of 30 subjects randomized and completed. As a result of randomization, 16 subjects received the placebo and 14 subjects received lubiprostone. The lubiprostone and placebo groups were similar except that the lubiprostone group had a somewhat lower baseline MTV of Ensure.

Effect of Lubiprostone on Gastrointestinal and Colonic Transit

Lubiprostone retarded gastric emptying (Table 3) compared with placebo, but it accelerated small bowel transit time (\( P = 0.017; \) Fig. 1). Additionally, colonic transit was significantly accelerated at 24 h (\( P = 0.033 \)) and, at 48 h, was accelerated at a rate that approached statistical significance (\( P = 0.084; \) Fig. 2).

Table 2. Demographics and other baseline data

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Lubiprostone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td><strong>Age, yr</strong></td>
<td>29.2 ± 1.9</td>
<td>33.1 ± 2.1</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>25.7 ± 0.9</td>
<td>25.8 ± 0.9</td>
</tr>
<tr>
<td><strong>Baseline MTV, ml</strong></td>
<td>1,268 ± 79</td>
<td>1,065 ± 84</td>
</tr>
<tr>
<td><strong>Baseline 30 min postsatiation, mm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nausea</strong></td>
<td>28.9 ± 6.3</td>
<td>18.6 ± 5.9</td>
</tr>
<tr>
<td><strong>Fullness</strong></td>
<td>67.5 ± 5.4</td>
<td>65.3 ± 4.2</td>
</tr>
<tr>
<td><strong>Bloating</strong></td>
<td>42.1 ± 7.9</td>
<td>27.2 ± 6.2</td>
</tr>
<tr>
<td><strong>Pain</strong></td>
<td>11.8 ± 4.7</td>
<td>8.1 ± 3.3</td>
</tr>
<tr>
<td><strong>Aggregate score</strong></td>
<td>150.2 ± 17.8</td>
<td>119.2 ± 12.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of subjects. BMI, body mass index.
Effect of Lubiprostone on Gastric Volumes

Lubiprostone increased fasting gastric volume \((P < 0.05)\); however, there was no significant effect on postprandial gastric volume (Fig. 3).

Effect of Lubiprostone on MTV and Postchallenge Symptoms

MTV values were reduced compared with placebo (Table 3) after adjusting for baseline. However, there was no significant change compared with baseline measurements. Although it appeared that lubiprostone reduced the MTV of a fully satiating meal, the volumes ingested by 13 of 14 volunteers in the lubiprostone treatment group were above the 5th percentile of the normal range of MTV (625 ml) previously identified in a separate study of adult healthy controls (14).

In contrast, 30 min after the completion of the fully satiating meal, there were no significant effects on postprandial symptoms after adjusting for multiple comparisons except for the symptom of fullness \((P < 0.01)\), which was lower with lubiprostone than placebo (Table 3).

Adverse Events

Adverse events (AEs) observed during this study were primarily gastrointestinal and were anticipated based on the action of lubiprostone and prior clinical trial data in larger samples of patients with constipation (25–27). The most common AEs reported by placebo and lubiprostone groups during the study were nausea (2 and 7 events, respectively), diarrhea (2 and 5 events, respectively), loose stools (2 and 4 events, respectively), and abnormal bowel sounds (0 and 2 events, respectively). Only one volunteer discontinued due to an AE; one participant experienced severe nausea after a single dose of placebo and subsequently withdrew from the study. All remaining AEs either occurred in only one subject or at a frequency less than or equal to placebo subjects.

DISCUSSION

This study demonstrates that the novel ClC-2 activator lubiprostone retarded gastric emptying but accelerated small intestinal and colonic transit in healthy subjects. These results are consistent with the hypothesis that lubiprostone facilitates small intestinal and colonic transit. Given the effect on chloride secretion, it is possible that the effects on small bowel and colon transit may reflect increased luminal water content, which resulted in a change in transit. In addition to their presence in epithelia, volume-activated anion currents have also been described in human and canine jejunal circular smooth muscle (20, 21), and, hence, it is conceivable that there

Table 3. Summary of data posttreatment

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Lubiprostone</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>16</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Gastric emptying (t_{1/2}), min</td>
<td>106.1±5.5</td>
<td>132.4±5.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Ascending colon (t_{1/2}), h</td>
<td>15.0±1.6</td>
<td>13.6±1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Posttreatment MTV, ml</td>
<td>1,242±51</td>
<td>1,091±51</td>
<td>0.050</td>
</tr>
<tr>
<td>Posttreatment 30 min postsatiation, mm</td>
<td>13.3±3.9</td>
<td>16.0±4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Nausea</td>
<td>68.5±3.1</td>
<td>55.4±3.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Fullness</td>
<td>41.6±5.0</td>
<td>30.4±5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Bloating</td>
<td>12.2±3.4</td>
<td>12.2±3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Pain</td>
<td>137.0±9.7</td>
<td>114.3±10.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values for gastric emptying \(t_{1/2}\) are means ± SE, values for ascending colon \(t_{1/2}\) are least-squares means ± SE adjusted for gender and BMI, and values for posttreatment MTV and posttreatment 30 min postsatiation are least-squares means ± SE adjusted for gender, BMI, and the corresponding baseline value; \(n\), no. of subjects. NS, not significant. \(P\) values were determined by analysis of covariance.

![Fig. 1. Small bowel transit time (in min). Data are least-square-adjusted means by analysis of covariance (ANCOVA), adjusting for gender and body mass index (BMI). *\(P = 0.017\).](http://ajpgi.physiology.org/)

![Fig. 2. Colonic transit. Shown are the geometric centers (GC) at 24 and 48 h. Data are least-squares-adjusted means + SE by ANCOVA adjusting for gender and BMI. *\(P = 0.033\).](http://ajpgi.physiology.org/)

![Fig. 3. Fasting and postprandial gastric volumes in response to placebo and lubiprostone. Note the significantly greater fasting volume with lubiprostone relative to placebo treatment and the similar postprandial volumes. Data are least-squares (adjusted) means + SE by ANCOVA using gender and BMI as covariates. *\(P = 0.049\).](http://ajpgi.physiology.org/)
is a direct effect of the CIC-2 activator on motor function. It is unlikely, however, that lubiprostone exerts a primary motor effect because it has little to no affinity for EP and FP receptors (37). In contrast to the serotonergic 5-HT4 agonists tegaserod and renzapride, which accelerate ascending colon emptying (8, 32), lubiprostone accelerated overall colonic transit without significantly accelerating the rate of ascending colon emptying. This observation is consistent with an effect on the colon beyond the ascending region. The proximal colon could compensate to reabsorb the fluid load resulting from accelerated small intestinal transit, as shown by the high capacity for fluid reabsorption in the human proximal colon (18, 24). The observation of accelerated overall colonic transit suggests that lubiprostone may have an additional secondary motor effect in addition to its secretory effect on the colon beyond the ascending region. Thus despite the potential for reabsorption of intraluminal fluid in the proximal colon, which may reduce the effect that would result from small intestinal secretion, lubiprostone may have an additional effect on the more distal colon, resulting in acceleration of colonic transit. This hypothesis requires further study, but, if proven, it may suggest that lubiprostone can relieve constipation with low risk of significant loss of fluids.

In addition to diarrhea and loose stools, subjects who received lubiprostone also reported nausea more frequently compared with control subjects in clinical trials and in this pharmacodynamic study in healthy subjects. A previous study (25) of lubiprostone in constipated patients has shown the rate of nausea to be lower than in the present study in healthy volunteers. The observation of diarrhea or loose stools in healthy volunteers provides pharmacodynamic evidence suggesting the drug would be efficacious in the treatment of chronic constipation. Nausea may be attributable to delayed gastric emptying; however, small intestinal distension has also been associated with nausea (1). Therefore, we cannot exclude the possibility that increased intestinal secretion, which may distend the small intestine, contributes to the nausea, because this has not yet been investigated. Overall, aggregate postprandial symptom scores in the presence of lubiprostone were comparable with placebo, suggesting the drug does not affect gastric accommodation or sensation after a fully satiating meal. However, lubiprostone significantly decreased the symptom of fullness after the challenge of a fully satiating meal. The effect of lubiprostone on gastric emptying appears not to be associated with increased scores for any of the symptoms after the challenge of a fully satiating meal. On the other hand, it is conceivable that there may be a beneficial effect in patients with functional dyspepsia who have accelerated gastric emptying (4) or in those who develop excess postprandial fullness, given the observation that lubiprostone treatment reduced fullness after the fully satiating meal. Reduced fasting gastric volume may contribute to symptoms in a subgroup of dyspeptics (19). There was no aggravation of the postprandial symptoms with lubiprostone. The delay in gastric emptying with lubiprostone would be expected to reduce the impact of small intestinal distention caused by secretion resulting from the effect of the drug on epithelial water and electrolyte transport.

Although the role of CIC-2 in gastrointestinal sensory functions is unclear, Cl− channels do modulate afferent function in the ear and laryngeal mucosa and in proprioceptive afferents (12, 22, 31). The potential for lubiprostone to reduce visceral sensitivity by altering the function of visceral afferents is also worthy of further study. The effects of lubiprostone on nausea declined over time in the clinical trials, suggesting that the transient nausea does not negate the beneficial effect on colonic function. Our experimental data showed that postprandial symptoms in healthy individuals are not increased after food challenge; indeed, the sensation of fullness was significantly reduced.

In summary, lubiprostone has been previously shown to be efficacious in the treatment of chronic idiopathic constipation (25–27); our study shows that it accelerates small bowel and colonic transit in healthy subjects. Effects on gastric emptying of solids are not associated with significant increases in postprandial symptoms after a liquid nutrient challenge test. Further mechanistic studies of colonic motility and secretion will enhance our understanding of the pharmacological effects of selective CIC-2 activators in the human gastrointestinal tract. The current data demonstrate that activation of CIC-2 in healthy human volunteers results in acceleration of small bowel and colonic transit and provide further support for the potential use of lubiprostone for the treatment of patients with delayed colonic transit, and further mechanistic studies of colonic motility in patients with chronic constipation are warranted.

ACKNOWLEDGMENTS

The excellent secretarial support of Cindy Stanislav is gratefully acknowledged.

GRANTS

This study was supported in part by National Institutes of Health Grants RR-00585 (to the Clinical Research Center); RO1-DK-54681, RO1-DK-67071, and K24-DK-02638 (to M. Camilleri); and RO1-HD-38666 (to A. E. Bharucha). This study was funded by Sucampo Pharmaceuticals Incorporated.

REFERENCES


