Effect of oral CCK-1 agonist GI181771X on fasting and postprandial gastric functions in healthy volunteers

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CCK is a gastrointestinal hormone that is released in response to food intake or a role in stimulation of the vagal afferent pathway originating from the gastroduodenal mucosa, it is possible that, by acting on peripheral CCK receptors, CCK released may alter the function of the stomach during the postprandial period, thereby influencing postprandial symptoms, including satiation. It is unclear whether CCK also plays a physiological role in control of gastric functions in the fasting period.

Relaxation of the proximal stomach in the postprandial period occurs concurrently with an increase in plasma CCK, and this also coincides with the early phase of intestinal digestion (27). The relaxation response of the stomach is reportedly reduced in ~40% of patients with functional dyspepsia (16, 34). The role of CCK in development of dyspepsia is still under investigation. Feinle et al. (14) showed that symptoms induced by gastric mechanical distension in patients with functional dyspepsia were reduced by administration of the CCK antagonist dexloxiglumide.

GI181771X is a 1,5-benzodiazepine that reduces food intake in rats; this action is thought to be mediated by activation of peripheral CCK-1 receptors on vagal afferent fibers and on the pyloric sphincter (1). GI181771X is devoid of classic benzodiazepine properties, and it is a potent, full CCK-1 receptor antagonist.

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agonist, which exhibits no CCK-B receptor agonist activity. It is not as potent an agonist of CCK-1 receptors in vitro as the COOH-terminal CCK octapeptide CCK-8. However, unlike CCK-8, which requires parenteral administration, GI181771X is orally active.

The aims of this study were to compare effects of the four dose levels of GI181771X and placebo on gastric emptying, gastric volumes during fasting and after a meal, and postprandial symptoms after a fully satiating meal in healthy volunteers. A comparison of tablet with solution was done to investigate the effect of rate of drug absorption on gastric function responses.

MATERIALS AND METHODS

Study participants. Healthy volunteers between 18 and 50 yr of age and with a body mass index of 18–31 kg/m² were recruited during a 6-mo period (April–October 2002) from the local community by public advertisement. Exclusion criteria were pregnancy or breastfeeding; abdominal surgery other than appendectomy, cesarean section, or tubal ligation; positive symptoms on a validated abridged bowel disease questionnaire (35); history of eating disorder, psychiatric disease, or drug abuse; present or previous chronic gastrointestinal illness; any systemic disease or use of medications that could affect gastrointestinal motility, affect appetite, interfere with absorption, or interact with study medication; and known hypersensitivity reaction or idiosyncrasy to any benzodiazepines or drugs chemically related to the study drug or foods to be administered. The use of prescribed or over-the-counter medication within 7 days of the study was prohibited, except for stable doses (>1 mo before and during study) of an oral contraceptive, estrogen, or thyroxine replacement. Screening procedures were completed within 14 days before the 1st study day.

Pharmacology of GI181771X. The circulating time to 50% emptying (t½) of GI181771X in healthy humans is ~1–2 h for the solution and longer for the tablet formulation (1). The maximum time is 15 min–2 h for the solution formulation and is more variable for the tablet. There is no evidence of significant accumulation of the drug after 8 wk of dosing. The medication is partly absorbed across the dose range tested in this study, with ~92% being recovered in feces after oral administration (38). There is no appreciable enterohepatic circulation of the drug. Analyses of urine GI181771X concentrations confirm low systemic exposure (<1% excreted) after oral administration. Pharmacokinetic parameters [maximum serum concentration (C_max) and area under the curve (AUC)] are approximately proportional to the dose, and in one study blood concentrations of GI181771X were approximately two times lower in the presence of food. Single or repeated doses up to 24 mg/day were well tolerated by healthy overweight and obese subjects. The most common adverse events in phase I studies were dose limiting and included nausea, vomiting, diarrhea, abdominal cramping, tiredness, and headache.

Study design. This randomized, gender-stratified, parallel-group, five-arm, double-blind, double-dummy, placebo-controlled study was approved by the Mayo Foundation Institutional Review Board and informed consent was obtained from all participants. Randomization assigned each volunteer to one of four doses of GI181771X or placebo. The four doses of GI181771X were 0.1, 0.5, and 1.5 mg of solution and a 5.0-mg tablet. The solution was GI181771X or placebo in 5.0 ml of polyethylene glycol 400. The study medication was also provided in the form of 5.0-mg tablets with matching placebo. On each of the 3 study days, the participants received a 5.0-mg dose (via an oral dosing syringe) and a tablet that matched the 5.0-mg tablet formulation according to a randomization scheme kept at the research pharmacy. Subjects received the same dose on each of the 3 study days.

Participants received drug solution and a placebo tablet, placebo solution and a drug tablet, or placebo solution and a placebo tablet. Participants were given a glass of 60 ml of water after the solution formulation. This was standardized to avoid potential bias from volume ingested on the measurement of fasting and postprandial gastric volumes.

There were three dosing periods for each subject. Before administration of the study drug or placebo, fasting participants had a blood pregnancy test (if applicable) and alcohol/drug screening tests. Dosing occurred within 30 min before the start of each experimental study. Timing of administration was based on previous studies showing detectable plasma levels at 10 min and maximum time of 30 min for the liquid formulation administered orally.

All subjects were enrolled for assessment of scintigraphic gastric emptying of solids (study day 1), gastric volume measurement using the single photon emission computed tomography (SPECT) technique (study day 2), and postprandial symptoms using the satiety test (study day 3). At least 24 h lapsed between study days to allow for washout of study drug and test radioisotopes. The sequence of studies is shown in Fig. 1. Radiation exposure was within permissible limits for human volunteer studies and is documented in a previous publication from our laboratory (21).

Gastric emptying of solids. Gastric emptying of solids was measured using the scintigraphic method, which has been validated and reported previously (10). Briefly, fasting volunteers presented to the research unit, and a blood pregnancy test was performed (when applicable) within 48 h of the test. The participant took the study medication or placebo with a glass of water (200 ml) at 30 min before the radiolabeled meal. 99mTc-sulfur colloid (0.75 mCi) was added to two raw eggs during the scrambling, cooking process. The eggs were served on one slice of buttered bread along with a 240-ml glass of 1% milk (296 kcal total calories, 32% protein, 35% fat, 33% carbohydrate). Anterior and posterior gamma camera images were obtained immediately after meal ingestion, every 15 min for the first 2 h, and then every 30 min for the next 2 h (for a total of 4 h after the radiolabeled meal) to assess gastric emptying. A standardized meal (550 kcal: chicken, potato, and pudding) was given 4 h after ingestion of the radiolabeled meal.

Data were analyzed as described in previous studies (5, 6). Geometric mean of decay-corrected counts in anterior and posterior images of the stomach were used to estimate the proportion of 99mTc emptied at each time point (gastric emptying).

Measuring fasting and postprandial gastric volume. On study day 2, the second dose of study drug or placebo was administered ~5 min before intravenous injection of 99mTc-pertechnetate (TcO₄⁻) necessary for SPECT-based measurement of gastric volume. As described in previous studies from our laboratory (3, 22), tomographic images were acquired on a large-field-view dual-head gamma camera system (SMV SPECT System) equipped with low-energy, high-resolution collimators. Subjects were placed in a supine position on the imaging table with the detectors over the upper and midabdomen to ensure imaging of the stomach and small bowel. At 10 min after the intravenous injection of 10 mCi of 99mTcO₄⁻, dynamic tomographic acquisition was performed using the multiorbit mode of the system. In this mode, the system performed three complete 360° orbits at ~15 min per orbit. For each orbit, images were acquired every 6° at 3 s per image. After completion of the acquisition, orbits were summed to improve counting statistics. These orbits were then reconstructed
spaced backprojection (Ramp-Butterworth filter, order 10, cutoff 0.45; Nyquist) to produce transaxial images of the stomach. Imaging was performed once during fasting and twice after ingestion of a 300-ml Ensure (Ross Laboratories, Columbus, OH) drink through a straw for a total of 32 min, the time needed for two orbits for image acquisition. Stomach volume measurements were performed using the ANALYZE PC 2.5 (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) software system (18), which has been used previously in volumetric imaging studies (3, 22).

### Pharmacokinetic measurements
Blood samples were drawn from each subject on the gastric emptying and gastric volume study days for analysis of serum GI181771X. Simultaneously with the solid meal gastric emptying assessments, blood samples were drawn before the dose and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, and 4 h after the dose. Simultaneously with the gastric volume assessment, blood samples were drawn before the dose and at 5, 15, 25, 35, and 45 min and 1 and 1.25 h after the dose. Serum samples were analyzed for GI181771X on fasting gastric volume was statistically significant (P < 0.01; Fig. 2). With the 1.5-mg solution, gastric emptying t½ was 159 min (95% CI = 142–177), which represented a significant delay in gastric emptying compared with placebo (gastric emptying t½ = 116 min, 95% CI = 97–135, P < 0.01). The 5-mg tablet was associated with gastric emptying t½ of 149 min (95% CI = 131–168), which did not achieve statistical significance compared with placebo (P = 0.052). Plots of the mean gastric emptying curves with each dose of drug are provided in Fig. 3.

### Pharmacokinetic analysis
Noncompartmental pharmacokinetic methods (WinNonlin version 3.2, Pharsight, Cary, NC) were used to determine the pharmacokinetic parameters for each subject from the GI181771X serum concentration vs. time profiles. C max and the time to reach C max (t max) were obtained directly from the data. The area under the serum drug concentration vs. time curve up to t max was calculated by the linear trapezoidal method. In addition to assessing drug absorption rates with the traditional C max and t max, AUC sub0–4 h was included as an integrated measure of absorption (2, 7). The partial AUC of 0–4 h was reported for study day 1 to better compare solution with tablet formulations.

### Results

#### Volunteer demographics
Sixty-six volunteers were screened, 62 were randomized, and 1 was excluded after randomization because of ineligibility. Four were excluded because of ineligibility during the screening process. The remaining 61 participants enrolled in the study (Table 1). After randomization, 12 were assigned to placebo, 12 to the 0.1-mg solution, 13 to the 0.5-mg solution, 13 to the 1.5-mg solution, and 11 to the 5.0-mg tablet arm.

#### Effect on gastric emptying of solids
The overall group (treatment) effect of GI181771X was statistically significant (P < 0.01; Fig. 2). With the 1.5-mg solution, gastric emptying t½ was 159 min (95% CI = 142–177), which represented a significant delay in gastric emptying compared with placebo (gastric emptying t½ = 116 min, 95% CI = 97–135, P < 0.01). The 5-mg tablet was associated with gastric emptying t½ of 149 min (95% CI = 131–168), which did not achieve statistical significance compared with placebo (P = 0.052). Plots of the mean gastric emptying curves with each dose of drug are provided in Fig. 3.

#### Effect on gastric volumes
The overall group effect of GI181771X on fasting gastric volume was statistically significant (P = 0.036). Fasting gastric volume was significantly greater in the group that received the 1.5-mg solution (329 ml, 95% CI = 276–382) than in the placebo group (223 ml, 95% CI = 166–281, P = 0.035; Fig. 4).

### Table 1. Participant demographics and maximum tolerated volume

<table>
<thead>
<tr>
<th>GI181771X</th>
<th>Placebo (n = 12)</th>
<th>0.1-mg solution (n = 12)</th>
<th>0.5-mg solution (n = 13)</th>
<th>1.5-mg solution (n = 13)</th>
<th>5.0-mg tablet (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24±4</td>
<td>29±8</td>
<td>27±4</td>
<td>30±6</td>
<td>30±11</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>4/8</td>
<td>4/8</td>
<td>5/8</td>
<td>5/8</td>
<td>5/6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22±2.5</td>
<td>24±3.7</td>
<td>25±3.1</td>
<td>24±2.8</td>
<td>24±2.7</td>
</tr>
<tr>
<td>MTV, liters</td>
<td>1.32 (1.12–1.52)</td>
<td>1.19 (0.99–1.38)</td>
<td>1.30 (1.11–1.50)</td>
<td>1.31 (1.12–1.50)</td>
<td>1.37 (1.16–1.58)</td>
</tr>
</tbody>
</table>

Values are means ± SD for age and body mass index (BMI). maximum tolerated volume (MTV) data are means with 95% confidence intervals (CI) in parentheses. M, male; F, female.
The group effect on postprandial gastric volume was also statistically significant ($P = 0.015$), with the 1.5-mg solution group showing higher gastric volumes (908 ml, 95% CI = 849–967) than the placebo group (799 ml, 95% CI = 735–863, $P = 0.056$).

Effect on maximum tolerated volume and postprandial symptoms. The overall effect of GI181771X on maximum tolerated volume ($P = 0.75$; Table 1) or aggregate ($P = 0.37$) or individual symptom scores (Table 2) was not statistically significant among the study groups.

Pharmacokinetic results. The measure of overall drug exposure for GI181771X, AUC over 4 h (AUC$_{0-4}$), is shown in Table 3. AUC$_{0-4}$ for the 5-mg tablet is similar to AUC$_{0-4}$ for the 0.5-mg solution, indicating that the formulation delivers less drug than the solution formulation. Three measures of rate of drug absorption are shown in Tables 3 and 4: AUC$_{t_{max}}$, $C_{max}$, and $t_{max}$. In a comparison of tablet with solution, $t_{max}$ is ~20 min to 1 h longer for the tablet and $C_{max}$ is blunted for study days 1 and 2. The integrated parameter, AUC$_{t_{max}}$, shows good dose separation between the solution doses. The 5-mg tablet compares well with the 0.5-mg solution within a study day (Fig. 5).

Adverse events. The most common adverse events were emesis (6 subjects), headache (6 subjects), diarrhea (4 subjects), and nausea (3 subjects). The 1.5-mg solution group ($n = 13$) had the highest incidence of emesis and diarrhea: two subjects experienced emesis and two subjects had diarrhea. The highest incidence of headaches (4 subjects) was reported in the 5.0-mg tablet group ($n = 13$). One of the female participants became pregnant during the study. She had completed the gastric emptying and volume measurements before her positive pregnancy test. The radiation exposure to the fetus was estimated to be negligibly greater than background radiation. After careful follow-up by her obstetrician, pregnancy and delivery were normal, and the child had APGAR scores of 9 and 10 at 1 and 10 min, respectively, and is completely normal. No serious adverse events were reported during the study.

**DISCUSSION**

This study showed that the CCK-1 agonist GI181771X caused an expected delay in gastric emptying of solids, confirming its biological activity. The high-dose (1.5-mg) solution of GI181771X delayed gastric emptying significantly, in contrast to the lower solution doses. There is evidence of delay after the 5-mg tablet, but this did not reach statistical significance ($P = 0.052$). The increases in postprandial gastric volume and delay in gastric emptying are consistent with evidence that CCK relaxes fundic tone and stimulates pyloric contraction (15, 20).
Table 2. Postprandial symptom scores

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>0.1-mg solution</th>
<th>0.5-mg solution</th>
<th>1.5-mg solution</th>
<th>5.0-mg tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggregate symptoms</td>
<td>162 (110–213)</td>
<td>208 (158–257)</td>
<td>163 (113–212)</td>
<td>180 (133–227)</td>
<td>222 (168–275)</td>
</tr>
<tr>
<td>Nausea</td>
<td>30 (13–47)</td>
<td>51 (35–67)</td>
<td>32 (16–48)</td>
<td>41 (25–56)</td>
<td>56 (38–73)</td>
</tr>
<tr>
<td>Bloating</td>
<td>46 (28–65)</td>
<td>60 (42–77)</td>
<td>47 (29–65)</td>
<td>48 (31–66)</td>
<td>62 (43–82)</td>
</tr>
<tr>
<td>Fullness</td>
<td>71 (60–83)</td>
<td>67 (56–79)</td>
<td>68 (57–80)</td>
<td>71 (61–82)</td>
<td>82 (70–94)</td>
</tr>
<tr>
<td>Pain</td>
<td>14 (1–29)</td>
<td>31 (16–45)</td>
<td>15 (0.87–30)</td>
<td>20 (6.0–34)</td>
<td>22 (6–37)</td>
</tr>
</tbody>
</table>

Data are means with 95% CI in parentheses. Aggregate symptoms score was calculated at 30 min postprandially.

Fasting and postprandial gastric volume were significantly increased by the 1.5-mg solution, not by the other solution doses or the tablet formulation. These effects reflect different absorption characteristics and are evident in the AUCmax measurements, where AUCmax was highest for 1.5-mg dose, lower for the 0.5-mg solution dose and the 5-mg tablet (which showed similar average AUCmax), and lowest for the 0.1-mg solution dose. There was a trend for increasing gastric emptying delay with increasing doses in solution.

A comparison of tablet with solution pharmacokinetics and pharmacodynamics was done to investigate the hypothesis that the rate of drug absorption is most important to elicit pharmacological responses. Rate of drug absorption is believed to be important, in view of the location of the CCK-1 receptors on vagal afferent fibers and their relation to gastric function. Therefore, a faster rate of absorption or a higher dose would provide a higher concentration of GI181771X to the submucosal receptors and more drug effect on first pass through the intestinal absorptive membranes. The tmax is ~20 min to 1 h longer for tablet than solution, and Cmax is lower with tablet than solution for study days 1 and 2. The integrated parameter, AUCtmax, further supports the conclusion that drug is absorbed more quickly from the solution formulations than from the tablet, and this explains, at least in part, the different gastric function results with the 5-mg tablet formulation. Collectively, the pharmacokinetic results indicate that the absorption rate of GI181771X is more rapid from the solution than from the tablet formulation. The difference in pharmacokinetic parameters between study days 1 and 2 illustrates the sensitivity of drug absorption to the presence of food. On day 1, the participants ingested solid food during measurement of gastric emptying, whereas on day 2, they ingested exclusively liquid nutrient during measurement of gastric volumes. Body posture was different during the first hour of pharmacokinetic measurements on the 2 study days (supine during gastric volume measurements and seated during gastric emptying study). Differences in posture during the 1st h may also contribute in part to the different pharmacokinetics on the 2 study days.

Liddle et al. (25) reported delayed gastric emptying with exogenous CCK doses that aimed at mimicking postprandial levels: mixed meal (analogous to the solid-liquid meal in our study) and water emptying were delayed. The significant dose-related effect with the liquid formulation and the borderline effects of the 5-mg tablet formulation of the synthetic CCK agonist on retardation of gastric emptying of solids are consistent with those reported in the literature, and differences in activity clearly reflect the pharmacokinetics of the drug.

The effect on the postprandial volume seen in our study with the synthetic CCK agonist is also consistent with the well-documented role of endogenous CCK released in response to arrival of nutrients in the duodenum. For example, Feinle et al. (14) showed that intraduodenal lipid increased postprandial gastric volume, compliance, and plasma CCK levels. In their series of elegant studies, they showed that the CCK-1 antagonist dexloxiglumide abolished the increase in postprandial gastric volume induced by duodenal nutrients. By studying the CCK-1 agonist GI181771X, we have additionally been able to demonstrate that this agent increased fasting gastric volume while also demonstrating the known CCK effects on postprandial volume. Our observations on the effect of the CCK-1 agonist on fasting volume are novel and suggest that CCK-1 receptors may be activated to change fasting gastric volume, even in the absence of food-mediated endogenous CCK release from the duodenum.

Other studies using CCK infusion (37), cerulein, or the CCK-1 antagonist loxiglumide (4, 28, 39) failed to show changes in fasting gastric tone, compliance, or myoelectrical activity. However, in the previous studies, the exogenous CCK was infused at doses that mimicked postprandial endogenous CCK levels, which may not reflect tissue levels and may have been too low to activate these CCKergic mechanisms during fasting. In fact, lower esophageal...
sphincter pressure reduction by CCK in humans is critically dependent on the infused dose (23), and the steady-state levels in plasma observed with doses that reduced lower esophageal sphincter pressure far exceed the endogenous postprandial CCK levels (23). Moreover, the method used to measure gastric tone in previous studies did not appraise the entire stomach, in contrast to the SPECT imaging method used to measure volume in this study. The CCK-1 antagonist loxiglumide and its derivative dexloxiglumide, which were always efficacious in altering gastric tone in the postprandial period, may not be ideal pharmacological probes to assess whether CCK mechanisms can be stimulated during fasting when endogenous CCK levels are low. This novel CCK-1 agonist GI181771X provides a means to test whether such mechanisms can pharmacologically modulate gastric functions during fasting. This may be relevant to studies of satiation in obesity; a recent large study showed that fasting gastric volume is an important determinant of time to satiation after meal ingestion (12), with higher fasting gastric volumes being associated with a later time to satiation when calories are ingested at a constant rate. Conversely, the CCK-1 agonist may serve as a probe to test the hypothesis that increasing fasting gastric volume may decrease satiation and facilitate greater calorie intake in patients with early postprandial fullness as reported in patients with functional dyspepsia (34).

We observed no difference in the maximum tolerated volume or in the aggregate or individual symptom scores for nausea, bloating, fullness, or pain. In the study by Feinle et al. (14), there was an increase in symptoms of fullness and pain with infusion of 20% lipid in dyspeptic patients. However, in the study by Feinle et al., mechanical distension of the stomach with a balloon was used to induce symptoms while gastric relaxation was inhibited with the CCK-1 antagonist. Hence, the experimental design is quite different from our study, which used a more physiological stimulus (a fully satiating meal). Another major difference is that our study was performed in healthy controls, rather than in patients with functional dyspepsia.

It is worth emphasizing that the results of Feinle et al. (14) in healthy controls are consistent with those reported here in healthy participants.

Although the motor mechanisms altered by this CCK-1 agonist require further elucidation with antropyloroduodenal manometry, it is likely that the motor effects described with infusion of lipid into the duodenum reflect the effects of CCK and its endogenous stimulators. Thus Heddle et al. (19) demonstrated that intraduodenal lipid induced isolated pyloric pressure waves and reduced antral contractions and antropyloroduodenal coordination, all of which are potential mechanisms to delay gastric emptying of solids. Antral denervation studies in the rat suggest that antral neurons are not required for inhibition of gastric emptying by CCK (20). The enhanced reservoir function of the stomach reflected in fundic relaxation (14) or gastric volume increase (as demonstrated in this study) may also contribute to the delay in fundoantral delivery of food and onset of antral trituration and, ultimately, result in delayed emptying of solids from the stomach. The effects of GI181771X on pyloric tone and resistance to flow may be critical to the effects on emptying, as shown with CCK-8 (15), and require further study.

In conclusion, we have shown that GI181771X, a CCK-1 agonist, is biologically active. The 1.5-mg solution delays gastric emptying of solids and increases fasting gastric volumes. GI181771X exhibited an acceptable safety profile; predominantly gastrointestinal adverse events affected a minority of subjects. Effects during fasting suggest that CCK-1 mechanisms may be pharmacologically targeted to alter fasting gastric function, a previously unrecognized property of CCK-ergic mechanisms. Further studies are needed to clarify the effects and mechanism of action of GI181771X in patients with upper gastrointestinal disorders.

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