Effect of a glucagon-like peptide 1 analog, ROSE-010, on GI motor functions in female patients with constipation-predominant irritable bowel syndrome

Michael Camilleri,1 Maria Vazquez-Roque,1 Johanna Iturrino,1 Amy Boldingh,1 Duane Burton,1 Sanna McKinzie,1 Banny S. Wong,1 Archanja S. Rao,1 Enda Kenny,2 Maria Månsson,2 and Alan R. Zinsmeister3

1Clinical Enteric Neuroscience Translational and Epidemiological Research, College of Medicine, Mayo Clinic, Rochester, Minnesota; 2Rose Pharma A/S, Copenhagen, Denmark; and 3Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, College of Medicine, Mayo Clinic, Rochester, Minnesota

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Camilleri M, Vazquez-Roque M, Iturrino J, Boldingh A, Burton D, McKinzie S, Wong BS, Rao AS, Kenny E, Månsson M, Zinsmeister AR. Effect of a glucagon-like peptide 1 analog, ROSE-010, on GI motor functions in female patients with constipation-predominant irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 303: G120–G128, 2012. First published April 19, 2012; doi:10.1152/ajpgi.00076.2012.—The glucagon-like peptide 1 (GLP-1) analog ROSE-010 reduced pain during acute exacerbations of irritable bowel syndrome (IBS). Our objective was to assess effects of ROSE-010 on several gastrointestinal (GI) motor and bowel functions in constipation-predominant IBS (IBS-C). In a single-center, randomized, parallel-group, double-blind, placebo-controlled, dose-response study, we evaluated safety, pharmacodynamics, and pharmacokinetics in female patients with IBS-C. ROSE-010 (30, 100, or 300 μg sc) or matching placebo was administered once daily for 3 consecutive days and on 1 day 2–10 days later. We measured GI and colonic transit by validated scintigraphy and gastric volumes by single-photon emission computed tomography. The primary end points were half time of gastric emptying of solids, colonic transit geometric center at 24 h, and gastric accommodation volume. Analysis included intent-to-treat principle, analysis of covariance (with body mass index as covariate), and Dunnett-Hsu test for multiple comparisons. Exposure to ROSE-010 was approximately dose-proportional across the dose range tested. Demographic data in four treatment groups of female IBS-C patients (total 46) were not different. Gastric emptying was significantly retarded by 100 and 300 μg of ROSE-010. There were no significant effects of ROSE-010 on gastric volumes, small bowel or colonic transit at 24 h, or bowel functions. The 30- and 100-μg doses accelerated colonic transit at 48 h. Adverse effects were nausea (P < 0.001 vs. placebo) and vomiting (P = 0.008 vs. placebo). Laboratory safety results were not clinically significant. In IBS-C, ROSE-010 delayed gastric emptying of solids but did not retard colonic transit or alter gastric accommodation; the accelerated colonic transit at 48 h with 30 and 100 μg of ROSE-010 suggests potential for relief of constipation in IBS-C.

There is unmet need in the treatment of irritable bowel syndrome (IBS), particularly the relief of abdominal pain (10). The glucagon-like peptide 1 (GLP-1) analog ROSE-010 has been shown to reduce acute exacerbations of IBS in a clinical trial involving 166 patients (25). Patient-rated pain relief and intensity were measured on a 100-mm visual-analog scale. The primary efficacy variable was the proportion of patients with >50% maximum total pain relief response from 10 to 60 min after treatment. Twice as many patients were responders after 100- and 300-μg GLP-1 analog injections compared with placebo. Similar results were obtained for the proportion of patients with total pain intensity response. ROSE-010 is in clinical development as a treatment for IBS and dyspepsia, targeting abnormal motor activity in the gut.

GLP-1 is normally released after food intake; it stimulates insulin release and reduces gastric emptying and small intestinal motility (24, 36, 41). Multiple GLP-1 analogs initially developed to normalize blood glucose levels in patients with diabetes have been shown to be safe and well tolerated in healthy subjects and patients (27, 45). GLP-1 causes gastric relaxation to facilitate normal food intake without the development of postprandial symptoms, such as early satiation, fullness, or bloating (19). This gastric accommodation seems to be dependent on a nitrergic link, as previously suggested for the rat small intestine and human stomach (2, 41). In humans, GLP-1 inhibits small intestinal motility in healthy subjects and patients with IBS (26). A prior report documented marked prolongation of colonic transit in a patient with a neuroendocrine tumor that secreted GLP-1 and -2 (8).

Compounds with GLP-1-like activity are hypothesized to reduce disordered gastrointestinal (GI) motor activity in IBS patients and to suppress acute pain attacks (23, 25). Given the inhibition of gastric and interdigestive small bowel motility, it is important to discern whether this medication would deleteriously retard GI and colonic transit, if it were to be administered subcutaneously once daily, as in a clinical trial that demonstrated efficacy relative to placebo for the treatment of acute attacks of pain (25). In fact, the safety of the medication would be most relevant in patients with constipation-predominant IBS (IBS-C); in a prior study, delayed transit at 24 h [colonic geometric center (GC) by scintigraphy on a scale of 0–5 was <1.50, the 10th percentile for healthy volunteers] was detected in 17.8% of 118 patients with functional constipation or IBS-C. Delayed transit was less frequent in patients with diarrhea-predominant IBS (8%) or mixed-symptom IBS (6.7%), and accelerated transit (GC >3.86, the 90th percentile for healthy volunteers) at 24 h was 4.2% of that in IBS-C patients (33).

The study hypothesis was that ROSE-010, administered subcutaneously, reduces gastric emptying of solids and enhances gastric accommodation without retarding colonic transit in female patients with IBS-C.

Given the prior report that this GLP-1 analog was proven to be efficacious in the treatment of acute attacks of abdominal...
pain (with pain ratings >40 on a 100-mm visual-analog scale) in a formal, randomized, placebo-controlled, crossover-design clinical trial (25), the primary objective of the present study was to assess the dose-related effects of ROSE-010 on pharmacodynamics (GI and colonic transit and gastric accommodation) compared with placebo in patients with IBS-C. Secondary objectives were to assess pharmacokinetics and safety of ROSE-010.

METHODS

Design

We conducted a single-center, randomized, parallel-group, double-blind, placebo-controlled, phase IIA dose-response pharmacodynamic, pharmacokinetic, and safety study in female patients with IBS-C. The study was registered with ClinicalTrials.gov (ID no. NCT01056107) and was approved by the Mayo Clinic Institutional Review Board on 23 October 2009; data were collected at the Mayo Clinic (Rochester, MN). The administration of one dose per day was intended to replicate the anticipated use for the relief of acute attacks of pain, as in prior (25) and future clinical trials.

Participants

Female participants, aged 18–65 yr inclusive, had previous diagnosis of IBS according to Rome III criteria (32), as well as a normal rectal examination result on file within the past 2 yr or performed at screen to exclude the possibility of an evacuation disorder, such as high sphincter tone at rest, failure of perineal descent by rectal examination result on file within the past 2 yr or performed at diagnosis of IBS according to Rome III criteria (32), as well as a normal rectal examination result on file within the past 2 yr or performed at screen to exclude the possibility of an evacuation disorder. Women of child-bearing potential (those who had not experienced a bilateral tubal ligation, hysterectomy, or menopause) used an acceptable method of contraception during the study.

We elected to study patients with IBS-C because of the need to demonstrate that, in patients who might have reduced small bowel motility, such as reduced interdigestive migrating motor complexes (28) or slow colonic transit (13), the GLP-1 analog did not cause delay of small bowel or colonic transit, which may potentially induce relevant clinical effects. For example, Deiteren et al. (18) showed that a 1-point change in colonic GC at 24 h is associated with ~0.6-point change in stool form based on the 7-point Bristol stool form scale (31).

Exclusion criteria included women who were pregnant or breastfeeding, clinically significant abnormal physical examination or laboratory results, structural or metabolic diseases/conditions that affect the GI system or functional GI disorders other than IBS-C, and inability to withdraw medications that affect GI transit (e.g., laxatives, tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors, analgesic drugs including opiates, nonsteroidal anti-inflammatory drugs, and cyclooxygenase-2 inhibitors, GABAergic agents, and benzodiazepines) 48 h prior to the study.

Medication: ROSE-010

The chemical name for ROSE-010 is Val10-GLP, and it is identical to native GLP-1 [GLP-1-(7–37)], but position 8 has been substituted by valine. ROSE-010 is a synthetic, selective GLP-1 analog with affinity for the GLP-1 receptor, prolonged half-life, and enhanced metabolic stability. Clinical pharmacology information is based on studies reported for the identical drug, LY-307161, which was previously under development for the treatment of type 2 diabetes mellitus, protecting against proteolytic NH₂-terminal degradation by dipeptidyl peptidase IV. This results in a longer duration of action than native GLP-1. It is rapidly absorbed, with mean maximum concentration (Cₘₐₓ) at ~0.5 h postdose and mean terminal half time (T₁/₂) of <1 h. Increases in Cₘₐₓ and area under the curve (AUC) were proportional to dose; apparent total clearance of the drug from plasma after oral administration and apparent volume of distribution during terminal phase after noninvasive administration were independent of dose. The values for Cₘₐₓ appeared to be inversely correlated with body mass index (BMI).

The formulation used in this study is associated with immediate release, in contrast to the sustained release formulation, which has a longer time to Cₘₐₓ (Tₘₐₓ) of ~3.5 h (38) and was previously associated with irritation at the site of subcutaneous injection. Nausea and vomiting were consistent dose-related side effects of LY-307161 (doses ≥0.5 mg produced nausea and/or vomiting in the majority of subjects). The maximum tolerated dose was, therefore, determined to be 400 μg. Electrocardiogram, spirometry, biochemistry, hematology, and urinalysis showed no relevant changes (38, 42). ROSE-010 is expected to be as safe as LY-307161 in clinical trial subjects.

Given the relatively rapid clearance of the immediate release formulation of this medication [mean Cₘₐₓ ~0.5 h postdose and mean terminal T₁/₂ <1 h], it might be anticipated that its effects on motor function would be more prominent in functions such as gastric accommodation or emptying, which occur over 0.5–4 h, in contrast to the more prolonged colonic transit, which occurs over 1–4 days in such patients. However, since it is anticipated that the medication will be administered daily, it was important in this study to assess its effects on colonic transit when it was administered daily for 3 days.

Randomization and Experimental Protocol

Participants were randomized to receive one of the doses; the randomization code was generated by computer and communicated to the research pharmacist. The allocation was concealed from all study participants and investigators. After data lockup on completion of the studies, the randomization code was provided to the study statistician.

ROSE-010 (30, 100, or 300 μg) or matching placebo was administered via abdominal subcutaneous injection once daily for 3 consecutive days and on 1 final day 2–10 days later, over a 13-day interval. Each of these days of ROSE-010 administration coincided with the dates when pharmacodynamic and pharmacokinetic studies were conducted, as summarized in the experimental protocol (Fig. 1). The gap between the different motility tests was required to allow for spontaneous isotope decay, to avoid interference by any residual radioactivity. The alternative approach would require laxative treatment, which might influence the motor functions being tested in response to the medication.

Measurement of GI Motor Functions

Validated GI and colonic scintigraphic transit and single-photon emission computed tomography (SPECT) measurements of fasting and postprandial gastric volumes were determined. The primary pharmacodynamic objective was to assess the dose-related effects of ROSE-010 on GI and colonic transit and gastric accommodation compared with placebo.

GI and colonic transit. An adaptation of our established scintigraphic method was used to measure GI and colonic transit. Briefly, 111In absorbed on activated charcoal particles was delivered to the colon by means of a methacrylate-coated, delayed-release capsule. The capsule was ingested following an overnight fast. Colonic transit was measured by means of the delayed-release capsule. After the capsule emptied from the stomach (documented by its position relative to radioisotopic markers placed on the anterior iliac crests), the study medication was given immediately before a standard radiolabeled breakfast meal was ingested. In this meal, 99mTc-sulfur colloid was used to label two scrambled eggs that were eaten with one slice of whole wheat bread and one glass of skim milk. This meal facilitates measurement of gastric and small bowel transit. Relative to the time of consumption of the breakfast meal, abdominal camera images were initially obtained every 15 min for the first 2 h and then every 30 min for the next 2 h. At 4 h, a standard lunch [chicken breast, potato, pudding, and milk (550 kcal)] was given. Camera images were taken...
Fig. 1. Experimental protocol. SPECT, single-photon emission computed tomography.

at 5, 6, and 8 h after the breakfast meal. At 8 h, the standard dinner [roast beef sandwich, sugar cookie, and milk (750 kcal)] was given. The patient returned on the following two mornings to receive study medication on both days, and 24- and 48-h scans were performed 15 min later. The performance characteristics of this test are summarized elsewhere (9, 12, 14, 15, 17, 18). For analysis of sequential images, a variable region of interest program was used to quantify the amount of radioactivity on anterior and posterior scans in the stomach and colonic regions. After correction for isotope decay and depth (using the geometric mean of anterior and posterior counts), gastric emptying, colonic filling curves, and colonic GC [weighted average of counts in 5 colonic regions, as in previous studies (9, 12, 14, 15, 17, 18)] were determined and end points (discussed below) were calculated for analysis of treatment effects. We previously showed that colonic filling at 6 h is a good surrogate measurement for small bowel transit time in healthy volunteers (28).

Assessment of stool frequency and consistency. During the study, patients completed a daily bowel pattern diary [which includes the 7-point Bristol stool form scale (31), ranging from 1 (for hard lumpy stool) to 7 (for watery diarrhea)] to record their bowel habits. The bowel pattern diary was dispensed at the screening visit, and the completed bowel pattern diary was collected at the completion of the study.

Assessment of gastric volume by 99mTc-SPECT imaging. A noninvasive SPECT method was used to measure gastric volume during fasting and 32 min after a liquid nutritional supplement (Ensure) meal (316 kcal, 300 ml). The method has been validated in detail elsewhere (9). In dyspeptic patients, the SPECT technique has reproduced the changes in gastric volume (29) reported using the barostat device. Changes in SPECT volumes have reproduced changes in gastric wall tone assessed with the barostat technique in response to pharmacological interventions, including GLP-1 (19). The performance characteristics of this method are summarized elsewhere (7).

Pharmacokinetic Assessments and Glycemic Indexes

Sample collection and handling. Blood samples for analysis of pharmacokinetic parameters were collected before dosing, at 10, 20, 30, 40, 50, 60, 90, 120, and 210 min postdose, and during visit 4 at 30 min postdose. The 10-ml sample, drawn into an EDTA collection tube, was centrifuged at 3,000 rpm for 15 min, and the serum was transferred to an anonymized polypropylene tube labeled only with a study number and patient initials as identifiers. The samples were frozen at −70°C until they were shipped in a batch to the laboratory (Quotient Bioresearch) for pharmacokinetic analysis. The total amount of blood drawn for these pharmacokinetic samples was ~90 ml.

Concentrations of ROSE-010 in the plasma samples were measured by liquid chromatography-mass spectrometry (LC-MS/MS), with subsequent pharmacokinetic analysis of concentration data. The method was validated at Quotient Bioresearch.

On blood samples taken at 15, 30 and 60 min postdose, we measured plasma glucose, glucagon, and insulin to assess glycemic responses to the GLP-1 analog.

Clinical and Laboratory Safety Assessments

A physical examination was performed at the screening visit. Weight and vital signs (including temperature, pulse, blood pressure, and respiration rate) were recorded at all study visits. Cardiovascular monitoring was indicated because of evidence that GLP-1 analogs affect blood pressure and heart rate (4, 44). Blood and urine samples were obtained for standard laboratory test safety assessments of hematology, blood chemistry, and urinalysis. Urine pregnancy tests were performed for women of child-bearing potential at screening and within 48 h prior to the receipt of radiation for the transit and SPECT tests.

Data and Statistical Analysis

Study end points. Primary end points were T1/2 of gastric emptying of solids (gastric transit), colonic GC at 24 h (colonic transit), and change between postprandial and fasting whole gastric volume (gastric accommodation). Secondary end points were gastric residual at 2 and 4 h, colonic GC at 4 h, colonic filling at 6 h, T1/2 of ascending colon emptying, colonic GC at 48 h, fasting and postprandial whole

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gastric volume, stool frequency and consistency, pharmacokinetic characteristics, and safety and tolerability of ROSE-010 administered as a subcutaneous injection.

Statistical power. Table 1 summarizes data for the primary response measures and uses the (relative) variation [coefficient of variation (CV%)] to estimate the effect size detectable with 80% power based on a two-sample $t$-test at a two-sided alpha level of 0.05. The effect size is the difference between means, detectable between treatment groups. CV, coefficient of variation; $T_{1/2}$, half time; SPECT, single-photon emission computed tomography; GC, geometric center.

<table>
<thead>
<tr>
<th>Response Type</th>
<th>CV, %</th>
<th>Effect Size Detectable ($n = 12$ per group) With 80% Power ($\alpha = 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric emptying of solids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{1/2}$ (min)</td>
<td>25</td>
<td>27% (30 min)</td>
</tr>
<tr>
<td>Fasting gastric volume (ml) by SPECT</td>
<td>37</td>
<td>39 (104 ml)</td>
</tr>
<tr>
<td>Postmeal gastric volume (ml) by SPECT</td>
<td>14</td>
<td>15 (112 ml)</td>
</tr>
<tr>
<td>Colonic transit GC at 24 h (range 1–5)</td>
<td>35</td>
<td>37 (0.81 GC units)</td>
</tr>
</tbody>
</table>

Effect size is the difference between means detectable between treatment groups. CV, coefficient of variation; $T_{1/2}$, half time; SPECT, single-photon emission computed tomography; GC, geometric center.

RESULTS

Participants

There were 46 female IBS-C participants; baseline demographic data in the four groups were not different. The patient disposition during the study is shown in Fig. 2. A total of 46 patients (not 48 patients, as planned) were randomized, because of the world-wide scarcity of $^{99m}$Tc for the transit and SPECT studies, which necessitated a 4-mo pause in the study and, eventually, time elapsed during which the study medication was stable and permissible for use according to regulatory guidance. One subject in the group receiving 100 µg of ROSE-010 and three subjects receiving 300 µg of ROSE-010 discontinued intervention; they were not lost to follow-up for safety and, in accordance to the statistical analysis plan, they were included in the intent-to-treat analysis using the prespecified imputation method (using the corresponding mean data value over all participants for the specific missing response value), with an adjustment in the ANCOVA error degrees of freedom by 4 (1 for each data value imputed).

The participants were equally distributed and well balanced for age, BMI, and baseline bowel movement frequency and consistency (Table 2) across the four treatment groups.

Pharmacokinetics

After subcutaneous administration of ROSE-010, maximum plasma concentrations were $0.311 \pm 0.247$, $0.924 \pm 0.343$, and $3.40 \pm 1.03$ ng/ml at 30, 100, and 300 µg, respectively (Fig. 3). $T_{max}$ was short, with median values of 0.33 h (range 0.33–3.5), 0.33 h (range 0.33–0.67), and 0.42 h (range 0.33–0.50) at 30, 100, and 300 µg, respectively.

The mean AUCs up to the last nonzero concentration (AUC<sub>0-tn</sub>) for the 3.5-h sampling period following dosing were $0.208 \pm 0.195$, $0.777 \pm 0.440$, and $3.16 \pm 0.896$ h·ng·ml<sup>-1</sup> at
30, 100, and 300 μg, respectively. The terminal T_{1/2} (where calculable) was short, with median values of 0.53 h (range 0.46–0.57) and 0.44 h (range 0.34–0.85) at 100 and 300 μg, respectively. Exposure (as measured by C_{max} and AUC_{0-\text{t}}) increased with increasing dose in a manner that was approximately dose-proportional at 30–100 μg (mean AUC_{0-\text{t}} increased from 2.08 to 0.77 h·ng·ml^{-1}) and was slightly greater than dose-proportional at 100–300 μg (mean AUC_{0-\text{t}} increased from 0.777 to 3.16 h·ng·ml^{-1}).

Exposure (as measured by C_{max} and AUC_{0-\text{t}}) increased with increasing dose in a manner that was approximately dose-proportional between 30 and 100 μg and was slightly greater than dose-proportional between 100 and 300 μg.

**Effects on Fasting and Postprandial Gastric Volume**

There were no significant effects of ROSE-010 on fasting and postprandial gastric volumes (Fig. 4) or on the change in gastric volume after the meal (data not shown).

**Effects on Gastric Emptying and Small Bowel Transit**

There was a significant treatment effect of ROSE-010 on gastric emptying of solids, as shown by T_{1/2} (P < 0.001; Fig. 5A, Table 2) and proportion of gastric emptying at 2 h (P < 0.001; Table 2). Thus, 100 and 300 μg of ROSE-010 significantly retarded gastric emptying T_{1/2} and proportion of gastric emptying at 2 h compared with placebo. Although there was an overall treatment effect on the proportion of gastric emptying at 4 h (ANCOVA P = 0.021), the results were most impressive for 300 μg, which differed from placebo (P = 0.0093).

There was no significant effect of ROSE-010 on colonic filling at 6 h (Fig. 5A).

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**Table 2. Participant baseline characteristics and primary and secondary response measurements**

<table>
<thead>
<tr>
<th>Group</th>
<th>Placebo (n = 12)</th>
<th>ROSE-010</th>
<th>ROSE-010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 μg (n = 11)</td>
<td>100 μg (n = 11)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>43.8 ± 2.3</td>
<td>43.4 ± 1.8</td>
<td>40.2 ± 3.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.7 ± 1.2</td>
<td>27.2 ± 1.2</td>
<td>26.4 ± 1.7</td>
</tr>
<tr>
<td>Mean no. of BMs/day at baseline</td>
<td>0.66 ± 0.11</td>
<td>0.65 ± 0.11</td>
<td>1.06 ± 0.23</td>
</tr>
<tr>
<td>Baseline stool consistency</td>
<td>2.40 ± 0.26</td>
<td>2.79 ± 0.34</td>
<td>2.97 ± 0.24</td>
</tr>
<tr>
<td>Gastric volume, ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>278.3 ± 14.2</td>
<td>287.6 ± 15.1</td>
<td>284.3 ± 18.9</td>
</tr>
<tr>
<td>Postprandial</td>
<td>307.4 ± 16.8</td>
<td>322.7 ± 20.6</td>
<td>330.9 ± 18.8</td>
</tr>
<tr>
<td>Proportion of GE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>0.41 ± 0.04</td>
<td>0.34 ± 0.04</td>
<td>0.24 ± 0.03d</td>
</tr>
<tr>
<td>4 h</td>
<td>0.88 ± 0.03</td>
<td>0.86 ± 0.03</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td>GE T_{1/2}, min</td>
<td>136.9 ± 7.2</td>
<td>151.8 ± 10.4</td>
<td>172.6 ± 7.1h</td>
</tr>
<tr>
<td>Colonic filling at 6 h, %</td>
<td>53.5 ± 4.8</td>
<td>47.6 ± 7.4</td>
<td>54.4 ± 7.8</td>
</tr>
<tr>
<td>Colon transit GC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>1.76 ± 0.14</td>
<td>2.39 ± 0.22</td>
<td>2.37 ± 0.41</td>
</tr>
<tr>
<td>48 h</td>
<td>2.67 ± 0.26</td>
<td>3.69 ± 0.23i</td>
<td>3.79 ± 0.33j</td>
</tr>
<tr>
<td>Ascending colon emptying T_{1/2}, h</td>
<td>19.3 ± 3.2</td>
<td>14.5 ± 1.6</td>
<td>14.8 ± 3.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANCOVA P < 0.001 for treatment effect; bP = 0.0075; cP < 0.001; dP = 0.0025; eP = 0.0001 vs. placebo by Dunnett-Hsu test.

ANCOVA P = 0.02 for treatment effect; fP = 0.0093 vs. placebo by Dunnett-Hsu test. hANCOVA P = 0.026 for treatment effect; iP = 0.032; jP = 0.018 vs. placebo by Dunnett-Hsu test.
Effect on Colonic Transit

Colonic transit at 48 h was accelerated by 30 and 100 µg of ROSE-010 (Fig. 5B). There were no significant effects of ROSE-010 on colonic transit at 24 h or T1/2 of ascending colon (Fig. 5B).

Bowel Function

There were no significant treatment effects on daily stool frequency or stool consistency (Fig. 6).

Safety and Adverse Effects

Adverse effects (Table 3) were nausea (8 of 11 subjects treated with 100 µg and 9 of 12 subjects treated with 300 µg; treatment effect \( P < 0.001 \) vs. placebo) and vomiting (2 of 11 subjects treated with 100 µg and 5 of 12 subjects treated with 300 µg; treatment effect \( P = 0.008 \) vs. placebo). No participants experienced constipation. Laboratory safety results were unremarkable (data not shown). There were no clinically significant treatment effects on glycemic indexes; Table 4 summarizes the number of values outside the normal range in each treatment group.

DISCUSSION

This study, conducted in 46 patients with IBS-C, showed that, as expected, the GLP-1 analog ROSE-010 delayed gastric emptying of solids, with impressive delays particularly at 300 µg. However, ROSE-010 did not retard small bowel or colonic transit, nor did it alter gastric volume or accommodation; in addition, no participants experienced constipation. Given the generally inhibitory effects of GLP-1 on gastric motor function, it was important to check whether these motor inhibitory effects might inhibit colonic transit. This would be very relevant if ROSE-010 is to be used to treat abdominal pain in patients with IBS-C. Contrary to the study hypothesis, ROSE-010 actually accelerated colonic transit at 48 h and did not retard colonic transit at the primary colonic transit end point at 24 h.

The effect of ROSE-010 on inhibition of gastric emptying appears to show dose proportionality. The study was designed to demonstrate an overall drug effect and individual dose comparisons with placebo; therefore, we did not compare the effects of 30, 100, and 300 µg of ROSE-010. However, there are clear numerical differences and an apparent dose proportionality of the treatment effect on gastric emptying. The pharmacokinetics profile shows the high concentrations during the first 90 min after administration, corresponding to the period when the stomach accommodates to the ingested meal and solids are being triturated in the stomach (11); the retardation of gastric emptying contrasts with the lack of effect of the doses tested on gastric accommodation.

The retardation in gastric emptying with 300 µg of ROSE-010 in the absence of a significant treatment effect on fasting or postprandial gastric volumes is likely to be the cause of the nausea and vomiting. Alternatively, the 300-µg dose may stimulate GLP-1 receptors, located within brain stem areas such as the area postrema, which regulates nausea. Importantly, the prevalence of emesis is much lower with the 100- than the 300-µg dose. The prevalence of nausea and emesis was relatively high in our study, which required repeated administration on 3 successive days and a fourth administration 1–7 days later during the SPECT study (Fig. 1). In contrast, in the study in which 128–134 patients received the same doses in a three-way, randomized crossover design (25), the prevalence of nausea and vomiting was 19% and 3%, respectively, with the 100-µg dose and 37% and 7%, respectively, with the 300-µg dose. As with other GLP-1 analogs, the repeated administration of the analog is sometimes associated with reduced prevalence of nausea and vomiting over time (40).

ROSE-010 did not significantly affect gastric volumes and accommodation. This appears to be contrary to the studies in the literature in which GLP-1 significantly increases gastric accommodation. We believe that the lack of effect may be dose-related, and, at the doses tested, there was no effect of this GLP-1 analog. Similarly, elevation of endogenous GLP-1 levels with the dipeptidyl peptidyl IV inhibitor vildagliptin was also not associated with an increase in gastric volume or accommodation (43).

There was no significant retardation of colonic filling at 6 h or colonic transit at 24 h; this may reflect the pharmacokinetics...
### Table 3. Adverse events

<table>
<thead>
<tr>
<th>Any adverse event</th>
<th>Placebo (n = 12)</th>
<th>30 µg (n = 11)</th>
<th>100 µg (n = 11)</th>
<th>300 µg (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Vomiting&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Dry heaving</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Stomach ache</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal cramp</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Flatulence</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Bloating</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lightheadedness</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Injection site rash</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Values represent number of individuals who experienced an adverse event.

<sup>a</sup>P = 0.063;  <sup>b</sup>P < 0.001;  <sup>c</sup>P = 0.047.

### Table 4. Number and proportion of participants in each treatment group with abnormal glucose, insulin, and glucagon levels

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Placebo</th>
<th>30 µg</th>
<th>100 µg</th>
<th>300 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;70 mg/dl</td>
<td>1 (0.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt;140 mg/dl</td>
<td>2 (1.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glucagon</th>
<th>Placebo</th>
<th>30 µg</th>
<th>100 µg</th>
<th>300 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undetectable</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt;80 µg/ml</td>
<td>25 (18.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Placebo</th>
<th>30 µg</th>
<th>100 µg</th>
<th>300 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.6 µU/ml</td>
<td>3 (2.2)</td>
<td>4 (3.1)</td>
<td>9 (7.1)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>&gt;24.9 µU/ml</td>
<td>27 (19.4)</td>
<td>12 (9.2)</td>
<td>5 (3.9)</td>
<td>7 (5.7)</td>
</tr>
</tbody>
</table>

Values represent number of values outside normal range at any time point (n); percentage (n/total number of values available) is shown in parentheses. Blood samples were obtained at 15, 30, and 60 min postdose.

The effect of ROSE-010 on colonic transit was not significant at 24 h (P = 0.22 overall), but colonic transit was significantly accelerated at 48 h (overall P = 0.026, with adjusted P < 0.05 for 30 and 100 µg). There are, nevertheless, numerical differences (of ~0.6 GC units) between the colonic transit at 24 h with 30 or 100 µg compared with placebo. We believe that the lack of significance at 24 h may represent a type II error. In fact, a post hoc analysis showed that the statistical power based on the observed variation and sample sizes suggests ~50% power to detect an overall treatment effect on colonic transit at 24 h. Despite the lack of statistical significance, a ~0.6-point difference in colonic GC at 24 h would be expected to be associated with a ~0.35 unit difference in stool consistency on the 7-point Bristol stool form scale (31). The ~1.0 GC unit difference at 48 h between 30 or 100 µg and placebo was significant and would be expected to result in a 0.65 unit difference in stool consistency on the 7-point Bristol stool form scale (31). It is possible that administration of the medication at 48 h, 15 min before imaging, contributed to the acceleration of colonic transit at 48 h.

The GC at 48 h is a useful parameter in IBS-C, as it is associated with a smaller coefficient of variation and is not compromised by a ceiling effect in disorders associated with constipation. This contrasts with diarrheal diseases, where the maximum transit measurement, GC of 5, representing all isotopes in stool, is frequently observed; however, this is seldom encountered with constipation disorders (33, 37). For example, studies of the colonic prokinetic tegaserod in functional constipation showed accelerated colonic transit at 48 h, but not at 24 h (39).

ROSE-010 did not significantly accelerate ascending colon T<sub>1/2</sub> (P = 0.70), suggesting that the overall acceleration of colonic transit at 48 h may reflect an effect of ROSE-010 on distal colonic motor function. This hypothesis requires further study. The mechanism whereby ROSE-010 accelerates colonic transit is unclear. The acceleration appears to involve the distal colon, since ascending colon emptying is not significantly accelerated. It is conceivable that a GLP-1 analog may facilitate left colon emptying by inhibiting the excessive contractions in the left colon in IBS-C, as originally reported by Connell (16) and, more recently, by Hasler et al. (22). This
hypothesis is supported by the observation that GLP-1 acts in the enteric nervous system by decreasing the excitatory cholinergic neurotransmitter through presynaptic GLP-1 receptors that modulate nitric oxide release (1) or by increasing sympathetic activity (5). There is also some evidence that, in vitro, GLP-1 induces weak smooth muscle contraction (3), but the propulsive effects of such contractions were not investigated. In conscious rats, GLP-1 accelerates colonic transit via central corticotropin-releasing factor (CRF) and peripheral vagal pathways (34). Interestingly, the retardation of gastric emptying with ROSE-010 may reflect the effects of GLP-1 activation of central CRF and peripheral sympathetic pathways, as demonstrated in rats (35).

In contrast to the higher gastric emptying $T_{1/2}$ with 300 μg than with 30 and 100 μg (consistent with a linear dose response), the dose-response curve of ROSE-010’s effect on colonic transit is U-shaped, indicating the potential for additional effects of 300 μg in colonic transit. For example, it is conceivable that effects such as nitric oxide release or sympathetic activation with the highest dose tested result in more profound inhibition of peristalsis, which ultimately retards colonic transit. This is in contrast to the inhibition of excessive uncoordinated contraction, which is hypothesized as the mechanism whereby this GLP-1 analog facilitates colonic transit at 30 and 100 μg. It is conceivable that the patients with IBS-C randomized in the 30- and 100-μg groups coincidentally had faster transit that was unrelated to the effects of the medication. We perceive this to be unlikely, given the observation that colonic transit was accelerated in only ~4% of 118 patients with IBS-C/functional constipation (33). Overall, the potential for GLP-1 to accelerate colonic transit via central CRF and peripheral vagal pathways, as reported in rats (34), is a hypothesis worthy of further study.

The stool frequency and consistency data did not show a statistically different treatment effect, although there was an increase in the number of stools per day in the group randomized to 100 μg of ROSE-010 per day. The lack of clinically demonstrable change in bowel function may reflect the short duration of treatment with ROSE-010. The single subcutaneous injections of 100 and 300 μg of ROSE-010 and placebo administered in a crossover design in the randomized, controlled trial by Hellstrom et al. (26) also did not document any change in bowel function as adverse events, although there was no prospective collection of bowel function information by questionnaire. Longer periods of ROSE-010 administration and formal collection of bowel function data are required to assess the potential role of ROSE-010 in the treatment of constipation in IBS-C. Our study did not require patients to be experiencing pain during the pharmacodynamic studies; hence, we did not attempt to evaluate the drug’s effects on pain, which have been reported in the literature (25).

We extensively evaluated the potential of this GLP-1 analog to affect postprandial glycemic control and the responses of glucagon and insulin; this is most relevant in the context of the proposed treatment of IBS patients who do not have abnormal glycemic control. The greater number of glucose levels below the lower limit on higher doses of ROSE-010 and greater numbers of insulin measurements above the upper limit with placebo or low-dose ROSE-010 reflect the effects of GLP-1 and its analogs in ameliorating postprandial glycemic control. The reduced blood glucose is not clinically important, as shown by the almost equal numbers of patients in each group with plasma glucagon levels above the normal limit. It is conceivable that ROSE-010 may have additional benefit in patients with diabetes mellitus who also suffer IBS; there is epidemiological evidence that increased BMI (which predisposes to type II diabetes mellitus) is associated with increased risk of GI symptoms, including IBS (20, 30).

The strengths of our study include the validated methods, intent-to-treat analysis, and testing of the medication in a dose range and frequency that mimic the anticipated administration in late-stage clinical trials. A minor weakness is the randomization of 46 patients (rather than 48 patients, as planned) before expiration of the shelf-life of the experimental medication.

In summary, these data support ongoing development of ROSE-010, especially the 30- and 100-μg doses, for relief of pain in IBS and, possibly, for relief of constipation in IBS-C. Our data also provide the first evidence in humans, confirming data in rodents (34), that a GLP-1 analog can accelerate colonic transit, with effects predominantly in the left colon. The degree of acceleration is unlikely to worsen diarrhea in patients with IBS with diarrhea and alternating bowel function, especially as the anticipated use of the drug will be intermittent, during acute pain associated with IBS. Further studies are required to clarify the motor mechanism that results in this acceleration of transit.

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DISCLOSURES

E. Kenny and M. Månsso, who are employees of Rose Pharma A/S, were involved in the pharmacokinetics analyses of the study. M. Camilleri, the corresponding author, had full access to all the data and takes full responsibility for the veracity of the data and statistical analysis.

AUTHOR CONTRIBUTIONS

M.C. is responsible for conception and design of the research; M.C. and M.V.-R. interpreted the results of the experiments; M.C. prepared the figures; M.C. drafted the manuscript; M.C., M.V.-R., J.I., E.K., and A.R.Z. analyzed the data. M. Camilleri, the corresponding author, had full access to all the data and statistical analysis.

REFERENCES


