Supplementary Materials and Methods

Evidence for the Use of Currently Available Prokinetics

The evidence for the use of currently available prokinetics is based on trials performed 2 or 3 decades ago and not on rigorous large trials using validated patient-response outcomes such as the GCSI[1] and Patient Assessment of Upper Gastrointestinal Disorders Quality of Life[2] that are now recommended by the clinical practice guideline of a national organization.[3] Metoclopramide is the only prokinetic therapy that currently is approved by the Food and Drug Administration, and a black box warning[4] recommends that its use should be limited to no more than 3 consecutive months because of the known risk of tardive dyskinesia and other extrapyramidal side effects,[5] even though the risk of irreversible neurologic adverse effects has been grossly overestimated.[6] Alternative prokinetic agents are domperidone (available under a special program by the Food and Drug Administration)[7] and erythromycin, which may show decreased clinical response after 4 weeks.[8] All 3 agents can be associated with cardiac arrhythmias that are associated with electrocardiogram QTc prolongation, especially in patients with *CYP2D6* polymorphisms resulting in poor metabolism of the drug and higher tissue levels.[9] There also may be clinically important drug interactions of erythromycin with CYP3A4 substrates such as benzodiazepines, neuroleptics, and 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors,[10] and medications that inhibit CYP3A may increase plasma erythromycin concentrations, thereby increasing the risk of ventricular arrhythmias and sudden death relative to a control antibiotic, amoxicillin.[11]

Pharmacokinetics of RM-131

In a randomized, placebo-controlled, single-ascending dose trial of 36 healthy male volunteers over a dose range of 3 to 2400 μg of RM-131, pharmacokinetic assessment of RM-131 showed the median time at which the peak plasma concentration is obtained after administration of drive (T_{max}) was 0.74 hours (range, 0.27–1.02 h), and the mean $t_{1/2}$ for elimination ranged from approximately 5 to 19 hours. RM-131 time after administration of t drug when the peak plasma concentration is reached (C_{max}) and the area under the curve increased proportionately with dose, and safety assessments suggested that RM-131 was well tolerated at all dose levels. Pharmacodynamic evaluations showed acceleration of GE with RM-131. Significant changes were seen at doses of 10 μg or greater, with the maximal effect seen at the 100-μg dose level with a GE $t_{1/2}$ change of -47.7 minutes or a 55% decrease (Rhythm Pharmaceuticals, data on file).

Eligibility and Identification of Patients for Participation

Diagnosis of diabetic gastroparesis was defined by the presence of the following: (1) at least a 3-month past or current history of upper GI symptoms (eg, postprandial nausea/vomiting, postprandial fullness, early satiety, anorexia, bloating, epigastric or abdominal pain); and (2) previously documented DGE by scintigraphy using a radiolabeled egg meal or by GE breath test using ^{13}C-spirulina platensis within the past 10 years. DGE by scintigraphy was defined as more than 60% retention at 2 hours or more than 10% retention at 4 hours. DGE by breath test was defined as kPcD (percentage dose excretion) values below the lower limit of normal at a minimum of 3 time points (45, 150, and 180 minutes).[12] No minimum symptom severity score was required for study eligibility and symptom assessment was not a primary end point.

Patients were identified by review of the Mayo electronic medical records, confirming a diagnosis of T1DM and documented DGE. A recruitment letter was sent to each potential participant's home, and those expressing interest were invited for screening. After approval by the Mayo Clinic Institutional Review Board and after signed written informed consent and confirmation of patient study eligibility, participants were enrolled by the blinded study coordinator (I.B.) and medical staff (A.S. and M.C.).

Experimental Protocol

After consent, patient study eligibility was confirmed by medical history, physical examination, concomitant medication review, clinical laboratory tests, and a 12-lead electrocardiogram. Medical records and history on evaluation were reviewed for prior evidence of diabetic complications such as peripheral neuropathy, retinopathy, or nephropathy. The absence of normal sinus arrhythmia (determined by assessment of the RR [R wave to R wave] interval on the baseline electrocardiogram by 2 investigators, A.S. and M.C.) was used as an indicator of cardiovagal dysfunction. Medications with potential cardiac effects were not discontinued and we did not use a respiratory frequency of 6 per minute because the presence or absence of cardiovagal dysfunction was not part of the eligibility criteria and evaluation of cardiovagal dysfunction was not a predefined end point. The use of sinus arrhythmia as an index of vagal function is well established in the literature.[13] All patients recorded GCSI-DD[14] during screening. Male and female patients between the ages of 18 to 65 years, meeting specific eligibility criteria, were enrolled in the study.

Eligibility criteria. All patients had an established diagnosis of T1DM, met criteria for symptoms consistent with diabetic gastroparesis as described earlier with prior documentation of DGE of solids, had previous exclusion of upper GI mechanical obstruction, had a glycosylated hemoglobin level of less than 10.1%, and had a body mass index of 18 to 40 kg/m^2. Women were required to be post-menopausal, surgically sterile, or nonpregnant and using an acceptable form of birth control. Men were required to agree to abstinence or use an acceptable form of birth control throughout the study.

Study procedure. On day 1 of period 1, patients reported to the CRU after an overnight fast for study drug dosing and subsequent pharmacodynamic and safety evaluations. Patients were allowed to take their usual morning study medications (except those for treatment of diabetes) with a sip of water on the morning of study drug administration. They were instructed to bring their insulin and glucometer to the study center on day 1 of both periods 1 and 2 and were instructed to self-administer their usual morning insulin injections, prorated based on meal caloric content. Fasting blood glucose was assessed before study drug administration and GE assessment to ensure relative euglycemia (goal, <275 mg/dL). Insulin treatment was administered to 1 patient on 1 treatment day to ensure the participant's blood glucose level was less than 275 mg/dL before ingestion of the radiolabeled meal. In a second individual, study procedures were initiated with a blood glucose value of 282 mg/dL. All other participants achieved the recommended cut-off value of less than 275 mg/dL.

Interventions. Patients received a single subcutaneous injection of study drug and received a standardized study meal 30 minutes after drug administration, which they were asked to consume within 10 minutes. The study meal consisted of 4 oz of scrambled EggBeaters (ConAgra Foods, Omaha, NE) that had been radiolabeled with 0.5 to 1.0 mCi of ^{99m}Tc sulfur colloid, 120 mL of water that had been radiolabeled with 100 μCi of ^{111}indium diethylene triamine pentaacetic acid, and 2 slices of white bread with strawberry jam. By using a solid-liquid radiolabeled meal and scintigraphy, we assessed GE over 4 hours and colonic filling at 6 hours. Gamma camera scans were obtained immediately after ingestion of the study meal through 6 hours after the meal. Patients were dismissed from the CRU after the final scintigraphic scan (6 hours after the meal). Safety evaluations included measurement of vital signs and assessment for adverse events on day 1. Patients also were contacted by telephone on day 2 to evaluate for adverse events. Concomitant medication review was conducted at screening and at each study visit.

After the washout period of at least 7 days, patients returned to the CRU in the fasted state for day 1 of period 2 dosing, following the same procedures as in period 1. After the follow-up telephone calls on day 2 of period 2, patients were terminated from the study.

Additional Statistical Analyses

A 2-tailed ($\alpha = .05$) paired t test (for GE at 1 and 2 hours, and GCSI-DD and NVFP scores, which were distributed normally) or Wilcoxon signed rank test (all other data) was used to compare end points based on the 2-period, 2-treatment cross-over design in 10 patients; the analysis was performed using the original randomly assigned treatment order. Only 1 GE data point was missing at 1 hour (for 1 subject at both visits), so we imputed a zero value (intent-to-treat conservative approach) and adjusted the P value for 8 degrees of freedom. Potential order effects were checked using a 2-sample test (2-sided $\alpha = .05$; eg, 2-sample t test or Wilcoxon rank sum test, as warranted), that is, comparing the within-subject treatment differences between those who received placebo first vs those who received active treatment first. Total adverse effects were compared using the McNemar test (a test for paired binary data), which appraised the difference between placebo and RM-131 in the proportions experiencing adverse effects. Given the use of 2 composite symptom scores (GCSI-DD and NVFP), we used the Hochberg[15] (step-up) approach to adjust the P values from the 2 paired t tests.

Additional Discussion: Potential Modifiers of Response to Ghrelin Agonist

Although this study was not designed specifically to assess potential modifiers such as cardiovagal dysfunction, a descriptive analysis was performed to show that RM-131 appears to be effective even in patients with cardiovagal neuropathy, suggesting that it may function at the stomach neuromuscular apparatus rather than through direct activation of the vagal nerve. A previous study in vagotomized patients showed that intravenous administration of ghrelin caused a significant increase in plasma growth hormone in patients, which was not significantly different from normal subjects.[16] Functional analysis of ghrelin-induced GI motility also has shown that there are 2 main mechanisms of ghrelin-induced responses.[17] In addition to activation of vagal afferent nerve terminals, direct activation of ghrelin receptors in the stomach and duodenum in response to peripherally administered intravenous ghrelin has been shown in vagally denervated rats.[18] The presence of cardiovagal dysfunction is highly suggestive of abdominal vagal dysfunction, especially in a long nerve such as the vagus and a demyelinating process such as diabetic neuropathy, and has been shown previously to be a good screening tool for abdominal vagal dysfunction.[19] Further study of RM-131 in patients with thoroughly documented cardiovagal dysfunction will be required.

References

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Supplementary Table 1. Summary of AEs in All 10 Patients in the Randomized, Cross-Over Study

	Placebo, N	RM-131, N	Severity as described by participant	Relation to study medication
Any adverse event	7	9	-	Possible
Hyperhidrosis	0	2	Moderate	Possible
Fatigue	1	1	Mild to moderate	Possible
Abdominal pain	1	0	Severe	Unlikely
Irritation at injection site	0	1	Mild	Likely
Hunger[a]	0	5	Mild to moderate	Possible
Shakiness	0	1	Moderate	Possible
Euphoria	1	0	Moderate	Unlikely
Hyperglycemia	0	2	Moderate to severe	Possible
Hypoglycemia	1	0	Mild	Possible
Burning in feet	1	0	Moderate	Unlikely
Flank pain	1	0	Mild	Possible
Abdominal pressure	0	1	Mild	Possible
Flatulence	1	0	Mild	Possible
Borborygmi	1	0	Mild	Possible

NOTE. The total number of AEs are shown.

[a]$P = .0625$; all others, $P =$ not significant. Comparisons were performed using the McNemar test.