DR M SCOTT HARRIS (Orcid ID : 0000-0001-5141-5773)

Article type : Original Article

The Effects of Ulimorelin, a Ghrelin Agonist, on Liquid Gastric Emptying and Colonic Transit in Humans

Short Title: **Ulimorelin Enhances Gastric Emptying**

Joyce James[1], Stuart Mair[2], Walter Doll[3], Erik Sandefer[3], David Wurtman[1], Alan Mauer[4], Adam M Deane[5], and M Scott Harris[1*]

[1] Lyric Pharmaceuticals, Inc., 601 Gateway Blvd, Suite 1020, South San Francisco, CA, 98040, USA; [2] Quotient Clinical Limited, Trent House, Mere Way, Ruddington, Nottingham, NG11 6JS, UK; [3] Scintipharma, Inc., 1721 Maywick View Lane, Lexington KY, 40504, USA;[4] Lewis Katz School of Medicine at Temple University, Philadelphia PA 19140; [5] Intensive Care Unit, Royal Melbourne Hospital, University of Melbourne, Melbourne, Victoria, 3050, Australia

*Corresponding author contact information: M. Scott Harris, (703) 431-3010, harris@middleburgconsultants.com

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/NMO.13784

Conflict of Interest Statement: Joyce James, David Wurtman and M. Scott Harris were employees of Lyric Pharmaceuticals. Alan Mauer and Adam Deane were consultants to Lyric Pharmaceuticals. Walter Doll and Erik Sandefer are employees of Scintipharma, Inc. Stuart Mair is an employee of Quotient Clinical Limited.

Author Involvement in the Study: Joyce James, David Wurtman and M. Scott Harris designed the study and composed the initial manuscript. Stuart Mair, Erik Sandefer and Walter Doll oversaw the conduct of the study, while Alan Maurer and Adam Deane advised on study design. All authors had access to the study data and reviewed and approved the final manuscript.

Source of Funding. This study was funded by Lyric Pharmaceuticals, 601 Gateway Boulevard, South San Francisco, California 94080. The study sponsor was primarily responsible for the study design, collection, analysis and interpretation of the data.
Keywords. ulimorelin, ghrelin, gastric emptying, colonic transit, prokinetic, α-1 acid glycoprotein

Abbreviations. AAGP: α-1 acid glycoprotein; AE: adverse event; AUC: area under the curve; C_{max}: maximum concentration; DG: diabetic gastroparesis; GH: grown hormone; GI: gastrointestinal; hGHS-R1a: human growth hormone secretagogue receptor 1a; IGF-1: insulin-like growth factor-1; IV: intravenous; MEC: minimum effective concentration; PD: pharmacodynamics; PK: pharmacokinetic; POI: post-operative ileus; Q8H: every 8 hours

Word count: 3899 (intro to conclusions; without abs, refs, figs, and tables); 2 tables, 2 figures, 1 supplementary table

Abstract (word count: 247)

Background. Ulimorelin, a small molecule ghrelin agonist and prokinetic agent, was effective in animal models of gastroparesis and delayed transit. However, employing once daily administration, it failed in clinical trials of postoperative ileus (POI), a condition in which colonic motility recovers last. The aim of this study was to evaluate drug dosing and regional differences in drug activity between stomach and colon.

Methods. Gastric emptying was assessed by scintigraphy in healthy adults at single doses of 600 to 1200 μg*kg^{-1} and multiple doses of 80 to 600 μg*kg^{-1} Q8H for 7 days. Colonic motility was assessed by 7-region scinitigraphic analysis at a dose of 600 μg*kg^{-1} for 2 days. The primary endpoints were percent change in time to 50% (Δt_{50}) liquid gastric emptying on Days 1, 4, and 6 and the geometric mean center of colonic transit at 24 hours (GC_{24}). Plasma concentrations of free and total ulimorelin were measured for pharmacokinetic and exposure-response modeling.

Key Results. Ulimorelin 150 to 600 μg*kg^{-1} every 8 hours resulted in statistically significant improvements (Δt_{50} = 23% to 46% ($p < 0.05$)) in gastric emptying from baseline that were sustained through Day 6. However, no effects on GC_{24} were observed. Pharmacokinetic analyses suggested that the free concentrations of ulimorelin achieved in POI trials and dosing frequency may have been inadequate.

Conclusions and Inferences. Ulimorelin is a potent gastric prokinetic but lacks evidence of activity in the human colon, pointing to the stomach as the predominant site of action of ghrelin in humans; ClinicalTrials.gov NCT02993055.

Introduction

Ghrelin is a 28 amino acid peptide secreted from the stomach during fasting[1,2]. Under normal conditions it is secreted in a pulsatile fashion three times a day before typical meal times and declines postprandially[3]. It has been termed the "feeding hormone"[4] because it stimulates gastric emptying and appetite[5,6] Low ghrelin concentrations have been correlated with intolerance to enteral feedings in critical illness[7,8]. Because of these properties, there has been interest in the development of ghrelin agonists for the treatment of gastrointestinal dysmotility.

Ulimorelin (LP101, TZP-101), a synthetic macrocyclic agonist of the ghrelin receptor (human growth hormone receptor, hGHS-R1a), accelerates gastrointestinal motility in animals and humans[9,10]. The effects of ulimorelin on the upper GI tract were confirmed in animal models of upper gastrointestinal dysmotility and clinical trials of diabetic gastroparesis [11,12]. While potent effects were also demonstrated in animal models of lower GI dysmotility, including spinal cord transection, it failed to meet endpoints in two multicenter clinical studies in postoperative ileus (POI)[13,14,15].

Although POI represents diffuse disruption of motility in the stomach, small intestine, and colon, the disruption of colonic motility is greater than other organs and recovers last[16,17]. A recent study of another peptide ghrelin agonist, relamorelin, suggested little effect on colonic transit, despite the reported 3-fold greater potency than ghrelin[18].

Ulimorelin is more than 99% bound to α-1 acid glycoprotein (AAGP), an acute phase reactant in plasma[19]; free ulimorelin, the active moiety, is therefore approximately 100-fold lower in concentration than total drug. Only free drug is available to exert a pharmacological effect or to be cleared; thus high binding to AAGP decreases not only the concentration of drug available in plasma but the duration over which pharmacologically relevant concentrations persist. Further, AAGP concentrations are elevated in post-surgical patients[20], resulting in even greater reductions in free drug concentration and duration than under normal conditions.

In this study, we evaluated the relationship between free concentrations and the motility effects of ulimorelin in the stomach and colon of healthy volunteers and provide further evidence that the primary activity of ghrelin and its agonists in humans resides in the upper GI tract.

Methods and Materials

Gastric emptying was assessed in two companion healthy volunteer studies conducted at Quotient Clinical Limited (Nottingham, UK) and Scintipharma (Lexington, KY, USA) between June 2015 and February 2016. Colonic transit was assessed in a healthy volunteer study conducted at Scintipharma (Lexington, KY, USA) between October and

December 2016. All of the authors had access to the study data and had reviewed and approved the final manuscript.

Assessment of Liquid Gastric Emptying. Male and nonpregnant female healthy volunteers aged 18 to 55 years inclusive and body weight between 50 to 90 kg were enrolled. The initial study consisted of single 30 minute IV infusions of ulimorelin 600, 900, and 1200 $\mu g*kg^{-1}$ and multiple 30 minute IV infusions of 80, 150, and 300 $\mu g*kg^{-1}$ every 8 hours (Q8H) for 7 days, versus placebo, while the follow-on study consisted of multiple 30 minute infusions of ulimorelin 600 $\mu g*kg^{-1}$ Q8H for seven days versus placebo. Cohorts of 12 subjects were randomized 3:1 to ulimorelin or placebo by an unblinded statistician. Study drug administration was blinded. Subjects were excluded for a history of recent GI symptoms potentially associated with altered transit, including nausea, vomiting, diarrhea or constipation.

In both studies, subjects received a standardized low fat, high fiber dinner, consisting of approximately 5 g fiber and 15 g fat on the evening of admission. As ulimorelin is formulated for intravenous use, the emptying of a liquid meal consisting of a commercially available enteral formula was studied to support potential indications in the acute hospital setting. The following morning, after an overnight fast of at least 10 hours, subjects received an initial infusion of study drug (ulimorelin or placebo) over 30 minutes. Subjects ingested a liquid meal consisting of 250 mL of Ensure Plus® (Abbott Nutrition, Maidenhead, Berkshire, United Kingdom) containing 375 kcals, 15.6 g protein, and 12.3 g fat and radiolabeled with 4 MBq of technetium-99 diethylene triaminepentaacetic acid (^{99m}Tc-DTPA), a nonabsorbable gamma emitter, 15 minutes after the infusion of study drug had commenced. Single isotope anterior and posterior static images of the stomach, each of at least 50 sec duration, were acquired in the standing position at five minutes after the start of the radiolabeled liquid meal (time 0), continuing at five minute intervals up to at least 135 minutes. This procedure was repeated with the first dose of drug on the morning of Day 4 in the first of the two studies and with the first dose of drug on the mornings of Days 4 and 6 in the second. Data acquisition and analysis was blinded to treatment. Residual gastric radioactivity was

determined from the geometric mean of anterior and posterior counts, as previously described[21].

Assessment of Colonic Transit. Male and female healthy volunteers aged 18 to 55 years inclusive and body weight between 50 to 90 kg were randomized 3:1 to ulimorelin 600 $\mu g*kg^{-1}$ or placebo administered Q8H IV for two days. All subjects underwent three days of diet conditioning on a balanced diet. Subjects were likewise excluded for a history of recent GI symptoms.

After an overnight fast of at least 10 hours, subjects received the first dose of study drug and a standard breakfast consisting of 120 g scrambled liquid egg white, two slices of white toast, 30 g strawberry jam, followed by 120 mL water 15 minutes after the meal was consumed[22]. Subjects also ingested a radioactive dose of 100 µCi indium-111-labeled diethylene triaminepentaacetic acid (^{111}In-DTPA) in 300 mL water within five minutes of the meal. Subjects then underwent standing anterior-posterior scintigraphic imaging hourly from 0-6 h and at 8, 12, 24, 32 and 48 h post ingestion of the radiolabel. Colonic emptying was assessed as described previously using a 7-region analysis to determine the geometric mean center of the ^{111}In counts at defined time points[23]. Colonic filling at 6 hours post meal was employed to estimate the sum of gastric and small bowel transit.

Safety Assessments. Safety assessments included vital signs, ECGs, and routine laboratory assessments. Heart rate was monitored by telemetry and Holter monitoring over the time course of dosing.

Study Endpoints. The primary endpoint for gastric emptying was the percent change in time to 50% emptying (Δt_{50}) of a liquid test meal. The primary endpoint for colonic transit was the geometric center at 24 hours (GC_{24})[23]. Secondary colonic transit endpoints included the following: 1) the geometric mean center at 8 and 48 hours (GC_8 and GC_{48}); 2) half-life ($t_{1/2}$) of ascending colon emptying; 3) colonic filling at 6 hours (a measure of small bowel transit); and 4) percent retained in the colon at 8, 24, and 48 hours. Safety endpoints included adverse events, ECGs, and laboratory evaluations. Telemetry and Holter monitor were employed to record and capture electrocardiographic changes.

Study Medications. Ulimorelin was supplied as a 2 mg*mL^{-1} pH balanced 5% dextrose (D5W) solution which was further diluted in D5W for infusion (Patheon Italia SpA, Monza, Italy). D5W served as the placebo. Study drug and placebo presentation was identical and was prepared by an unblinded pharmacist that did not otherwise participate in the study.

Pharmacokinetics and Modeling. Sampling for AAGP and total and free ulimorelin plasma concentrations in healthy volunteers was performed to determine ulimorelin pharmacokinetic (PK) parameters ($t_{1/2}$, C_{max} (maximal concentration) and AUC (area under the curve) for up to 96 h after the last dose for single dose and multiple dose cohorts (through Day 7). Samples were stored at ≤ -70^{o}C until analysis.

The determination of AAGP concentrations in plasma and serum was performed using a validated assay based on the Randox turbidometric assay (Randox Cat. No. AG2472; LGC Limited, Fordham, UK). Total and free ulimorelin plasma bioanalysis was performed using validated liquid chromatography mass spectrometry (LC-MS/MS) methods, where the measurement of free concentrations followed rapid equilibrium dialysis (LGC Limited, Fordham, UK). PK parameter estimation for total and free ulimorelin was modeled using noncompartmental analysis with Phoenix WinNonlin (v6.3 or v6.4, Certara USA, Inc., St. Louis, MO, USA).

Exposure-response for gastric emptying (free C_{max} versus Δt_{50}) in healthy volunteers was modeled with Phoenix WinNonlin (v6.4, Certara USA, Inc., St. Louis, MO, USA). Improvements in gastric emptying were presented as positive values, while reductions were assigned negative values. A simple maximal agonist activity (E_{max}) model was used for exposure-response (Phoenix WinNonlin model 101; $E = (E_{max}*C)/(EC_{50}+C)$, where E_{max} is the maximum response, EC_{50} is the drug concentration that elicits 50% of E_{max}, and C is the drug concentration). The minimum effective concentration (MEC) was defined as the mean free C_{max} at which the maximal effects (Δt_{50}) of ulimorelin were achieved in gastric emptying studies under steady state conditions.

The steady state free ulimorelin plasma concentrations in POI patients in prior trials were modeled at the two doses employed (160 µg*kg^{-1} QD and 480 µg*kg^{-1} QD) in those

studies. One hundred and fifty-three (153) individual serum samples continuously stored at ≤-70°C collected from 64 individuals from a multicentre study in patients with medical and post-surgical critical illness[24] were assessed for AAGP concentrations using the assay noted above. The post-surgical population consisted of 23 samples from 11 patients. Individual t-tests indicated that the post-surgical population was not different from other populations with the exception of pneumonia, and therefore the data were combined for all populations except pneumonia and one patient with an unknown diagnosis. The final dataset consisted of 113 samples from 49 patients admitted for post-surgical, sepsis, cardiac, or other, including GI and neoplasm, reasons.

Free drug concentrations in POI patients were estimated by bucketing AAGP levels into concentration bins centered every 10 mg*dL^{-1} over 30 to 280 mg*dL^{-1} in Graphpad Prism (v6.05). The effective dose in patients was calculated from the dose administered in healthy volunteers as (dose*normal AAGP concentration)/AAGP bin center. Free maximum exposures in post-surgical patients were then estimated using the dose proportionality relationship for free ulimorelin in healthy volunteers at each effective dose level.

Statistical Methods. The sample size for the gastric emptying and colonic transit studies were based the results of similar studies in healthy volunteers[25,26]. Descriptive and inferential statistical analyses were performed in SAS (v9.4 or later). The adjusted mean differences, 95% CIs, and p-values for each active treatment versus pooled placebo group were determined. Results were interpreted as statistically significant if the p-value was <0.05 and the 95% CIs excluded 0.

Both the absolute change and the change from baseline in post-dose (Day 1, 4, and 6) versus baseline (Day -1) scintigraphic parameters were determined and analyzed using ANCOVA modeling. The model included treatment as a fixed effect and the corresponding predose value as a covariate. Treatment comparisons were made to estimate the difference in the changes from baseline between each active treatment and the pooled placebo group. The method was specified as Restricted Maximum Likelihood

and the denominator degrees of freedom for the fixed effect was calculated using the Kenward and Roger's method.

Protocol Approvals and Subject Consent. The studies were approved by their respective institutional review boards or ethics committees and conducted in accordance with the principles of Helsinki, GCP, and applicable European and United States regulations. Written informed consent was obtained from all subjects prior to study participation. The use of samples from the two critical illness studies was approved by the Institutional Review Boards overseeing those studies.

Clinical Trial Registry. The first of two gastric emptying studies was registered on ClinicalTrials.gov (NCT02993055) at the request of the Medicines and Healthcare Products Regulatory Agency of the United Kingdom. The other studies were not registered, as Phase 1 healthy volunteer studies are not part of standard trial registry requirements in the US and Europe.

Key Results

Subject Disposition. A total of 51 subjects were enrolled and dosed in the two gastric emptying studies. Thirty-nine (39) (25 male and 14 female) subjects received ulimorelin and 12 (9 male and 3 female) subjects received the matching placebo. 24 subjects were enrolled in the colonic transit study; 15 (12 male and 3 female) received ulimorelin and 8 (4 male and 4 female) received placebo. One subject withdrew in the ulimorelin group before dosing due to hives. The groups were balanced with respect to sex, age, height, weight, and body mass index.

Gastric Emptying. On Day 1, there was a marked dose-dependent acceleration in gastric emptying (Δt_{25} and Δt_{50}) following ulimorelin administration (Table 1). Reductions ranged from 45% to 54% for Δt_{25} and 34% to 46% for Δt_{50} at doses from 150 to 600 $\mu g*kg^{-1}$. These effects were sustained at the 600 $\mu g*kg^{-1}$ Q8H dose through Day 6. Improvements were statistically significant with the exception of Δt_{25} at 150 $\mu g*kg^{-1}$ on Day 4. While there was the appearance of slight down-regulation of gastric emptying

from Day 1 to Day 4, the effects on Day 4 were sustained through Day 6 at the 600 $\mu g*kg^{-1}$ Q8H dose.

Small Bowel Transit. There was a trend of greater colonic filling at 6 hours post meal, a marker of gastric and small bowel transit, in the ulimorelin group compared with the placebo group (24% versus 50%, p-value = 0.0512), consistent with ulimorelin's effects on gastric emptying.

Colonic Transit. No differences between ulimorelin 600 $\mu g*kg^{-1}$ and placebo were observed following Q8H administration over 2 days (6 doses) on the primary endpoint of colonic geometric mean center at 24 hours. Nor were there significant differences between groups in any of the secondary endpoints of colonic transit (Table 2).

Exposure Response Modeling of Gastric Emptying. Exposure-response modeling of gastric emptying based on free C_{max} predicted EC_{50} values of 0.62 and 1.1 $ng*mL^{-1}$ on Day 1 and Day 4, respectively, with corresponding E_{max} estimates for the reduction in time to 50% liquid gastric emptying (Δt_{50}) of 49% and 35% (Figure 1). Between Days 1 and 4, there was a slight decrease in E_{max} with a corresponding increase in EC_{50}, suggesting down-regulation, but overall drug effect was persistent and robust on both days. MEC was determined to be 2.5 $ng*mL^{-1}$, equivalent to the mean free C_{max} on Day 4 at 150 $\mu g*kg^{-1}$ Q8H and corresponding approximately to EC_{70}.

Pharmacokinetics and Modeling

Free plasma concentrations were generally ≤1% of total (bound and unbound) plasma concentrations, achieving total and free steady state C_{max} of 6000 $ng*mL^{-1}$ and 40 $ng*mL^{-1}$, respectively, at 600 $\mu g*kg^{-1}$. Total ulimorelin PK was less than dose proportional with both volume of distribution and clearance increasing with dose, consistent with saturation of AAGP and a higher free fraction as dose increased. In line with these findings, free ulimorelin was greater than dose proportional. The $t_{1/2}$ for total bound and unbound plasma ulimorelin was approximately 22 hours, while the effective $t_{1/2}$ for free ulimorelin was only 2 hours, with essentially nil free concentrations for the remaining parts of the

day. The 8 hour dosing interval resulted in an accumulation of free C_{max} of about 2.4-fold by the second dose which remained stable over the dosing period of 7 days.

AAGP concentrations in both healthy volunteers and patients followed a log-normal distribution. Mean AAGP concentrations in combined patient dataset were 2.6-fold higher than healthy volunteers and spanned a range of 0.9- to 5.5-fold of normal, reflecting its biological property as an acute phase reactant (Figure 2). When free steady state concentrations of ulimorelin were modeled using these AAGP concentrations, only 0% and 43% of patients at the doses used in the previously published studies in POI, 160 $\mu g*kg^{-1}$ and 480 $\mu g*kg^{-1}$ QD, respectively, were estimated to have achieved the MEC for gastric emptying determined in this study (Supplementary Table 1).

Safety. Ulimorelin was well tolerated at all doses tested and adverse events were balanced between treatment and placebo groups. Mild to modest reductions in heart rate from baseline were observed following single doses above 600 $\mu g*kg^{-1}$ ulimorelin, which were not accompanied by any effects on blood pressure. These observations corresponded to the end of the 30-minute infusion, were short-lived and predictable, and reversed with the rapid decline in free concentration following the end of the infusion without need for intervention. One subject reported mild dizziness that resolved rapidly. The average reduction in heart rate at 600 $\mu g*kg^{-1}$ (free C_{max} of 25 $ng*mL^{-1}$; 10-fold the MEC) was approximately 4%. However, at the supratherapeuic dose of 1200 $\mu g*kg^{-1}$, corresponding to a mean plasma free C_{max} of 120 $ng*mL^{-1}$ (50-fold MEC), the average reduction in heart rate increased to 23%. Thus, 600 $\mu g*kg^{-1}$ was deemed the highest safe dose administered on a Q8H schedule in healthy volunteers to minimize heart rate effects.

Discussion

The activity of ulimorelin in normal, healthy volunteers was assessed in the upper and lower GI tract using the highest safe dose for healthy volunteers on a Q8H regimen, and while it increased gastric emptying in the stomach, it failed to improve colonic transit. The underlying assumption was ulimorelin pharmacology is free C_{max} driven, as the free concentration is only transiently high enough to elicit an effect before being cleared. This hypothesis was supported by the observation that heart rate changes were correlated with

the peak of the infusion. Furthermore, in animal toxicology studies, the toxicity of drug was correlated to C_{max} at the peak of the infusion; with shorter infusion rates, and thus concomitant higher C_{max}, eliciting greater toxicity (unpublished observations).

In healthy adult volunteers, ulimorelin achieved maximum gastric emptying effects over the dose range of 150 to 600 $\mu g*kg^{-1}$ Q8H that were sustained over multiple days. Robust prokinetic effects were observed with ulimorelin administration, with approximately 50% or greater improvements in gastric emptying times at the highest plasma levels. Liquid gastric emptying was selected for evaluation in view of the likely use of an intravenous prokinetic agent in the acute hospital setting, were liquid formulas are utilized for nutritional support. Overall gastric emptying of a high-calorie liquid meal has been shown to be comparable to a standardized egg-white sandwich solid meal[27].

Free ulimorelin binds to the hGHSR-1a receptor, a GPCR which internalizes following binding and is susceptible to tachyphylaxis[28,29]. The hGHSR-1a receptor undergoes internalization following a brief (20 minutes) exposure to its endogenous ligand, ghrelin, and recovery to the cell surface takes approximately 3 to 6 hours[28]. This highlights the importance of intermittent infusions to minimize the risk of desensitization of hGHSR-1a[29,30] and supports a dosing interval of 8 hours to evoke receptor activation multiple times daily, while avoiding tachyphylaxis. The use of a Q8H dosing schedule permitted three receptor activation events per day, versus a single event expected with QD dosing. Receptor recycling and tachyphylaxis could explain the failure of the motilide agonist, ABT-229, in the treatment of gastroparesis and functional dyspepsia, which was shown to have high desensitizing potency relative to motilin in vitro and ex vivo in preclinical models and was dosed twice daily in clinical studies[30,31].

While there was the appearance of slight down-regulation of the gastric emptying effect between Days 1 and 4 in the upper GI study, the maximal effects of drug were relatively similar. Importantly, prokinetic effects persisted through Day 4 of ulimorelin administration at all doses (80 to 600 $\mu g*kg^{-1}$ Q8H) and through Day 6 at 600 $\mu g*kg^{-1}$ (the only dose evaluated at this time point), demonstrating that tachyphylaxis does not occur at this dose and schedule. Furthermore, the magnitude of these effects on all days

compared favourably to studies of liquid gastric emptying with other prokinetic drugs such as the $5HT_4$ agonists prucalopride and velusetrag in healthy volunteers[32] or cisapride, metoclopramide and erythromycin patients with gastroparesis[33,34], for which, collectively, the range of improvements was approximately 15% to 30%.

This study demonstrated that ulimorelin has potent and sustained effects on gastric emptying but suggests it may have minimal effects on colonic transit under the conditions employed. Combined with the results of a previous study of another ghrelin agonist, relamorelin, in healthy volunteers[18], we conclude that the effects of ghrelin in humans are predominantly in the upper GI tract, with minimal effects on colonic transit. POI is primarily a lower GI disorder, as reflected by the relatively greater delay in the recovery of the colon compared to the stomach and small intestine after laparotomy[16,17]. In prior POI trials, the dual primary endpoint was the time to intake of solid food and time to first bowel movement. Since the colon recovers last, time to first bowel movement occurs later, gating the primary endpoint. The failure to achieve the primary endpoints of these trials could have been explained by the relative lack of potency of ulimorelin in the human colon.

In prior trials[10,12,15] ulimorelin was observed to be safe and well tolerated. Over the dose ranges and total daily doses tested in the current studies, most of which were significantly higher than those administered to humans in prior development, ulimorelin was similarly observed to be safe and well tolerated. While dose-dependent and exposure-dependent heart rate slowing was observed, the finding was minimal up to 600 $\mu g*kg^{-1}$ Q8H, the upper limit of the therapeutic dose range. The reductions in heart rate with increasing doses were likely attributed to up-regulated vagal tone and were not unexpected based on the vagally mediated effects of ghrelin agonism[13,35,36,37].

Our analysis provides a potential explanation for the failure of the observed effects of drug in POI studies; the promotility effects of ghrelin are predominantly exerted in the upper GI tract, with no to little effects on the colon. Since the recovery from POI predominantly reflects recovery of colonic transit [16,17], little effect would be anticipated.

Alternatively, however, animal studies have suggested that ghrelin agonists like ulimorelin, may be active in the colon and could be therapeutic agents in conditions such as constipation in spinal cord injury[13]. While ghrelin receptors cannot be identified in the human colon, the effects of ghrelin or its agonists are said to be mediated by the sacral parasympathetic nerve plexus, similar to its effects on the vagus nerve[38]. In preclinical studies, ulimorelin increased colorectal propulsion following injection at high concentrations directly into the sacral spinal cord[14]. However, in humans, evidence that ulimorelin penetrates the central nervous system is lacking.

Finally, the prior failure in POI could have been related to the dose and schedule employed, which is not unexpected at 160 $\mu g*kg^{-1}$, where 0% of patients are expected to have achieved steady state free concentrations of 2.5 $ng*mL^{-1}$, the MEC for upper GI effects in healthy volunteers and which was clearly unable to improve colonic transit in that population. At 480 $\mu g*kg^{-1}$, 43% of patients are expected to have achieved the MEC for upper GI effects at least once daily, with about 50% of those patients (or 21% overall) reaching free concentrations of approximately 2-fold the MEC or more. Furthermore, the effective $t_{1/2}$ for free ulimorelin was only 2 hours, with essentially nil free concentrations for the remaining parts of the day. It is possible that the doses used in prior studies were not high or frequent enough to achieve efficacy.

The strength of this study is that the assessments of gastric emptying and colonic transit effects were performed in a controlled setting in which accurate analyses of these parameters could be assessed. This allowed detailed exposure-response modelling that allowed us to evaluate key pharmacokinetic and pharmacodynamic variables. Limitations to these studies include the use of healthy volunteers with normal gastric and colonic motility, the possible use of a liquid versus solid meal, and the inability to determine if a higher MEC for colonic transit exists. However, these studies do lay the basis for future studies of gastric motility in the hospital setting.

In conclusion, ulimorelin is a potent gastric prokinetic but lacks evidence of activity in the human colon, consistent with prior observations that the stomach is the predominant site of action of ghrelin in humans, a finding that might apply to other ghrelin agonists as well. The results of the current study support further investigation of the safety and efficacy of ulimorelin in conditions of abnormal upper GI motility and shed light on the development of ghrelin agonists for other indications.

Acknowledgement, Funding and Disclosures

This study was funded by Lyric Pharmaceuticals, 601 Gateway Boulevard, South San Francisco, California 94080.

Joyce James, David Wurtman and M. Scott Harris were employees of Lyric Pharmaceuticals. Alan Mauer and Adam Deane were consultants to Lyric Pharmaceuticals. Walter Doll and Erik Sandefer are employees of Scintipharma, Inc. Stuart Mair is an employee of Quotient Clinical Limited.

Author Involvement in the Study

Joyce James, David Wurtman and M. Scott Harris designed the study and composed the initial manuscript. Stuart Mair, Erik Sandefer and Walter Doll oversaw the conduct of the study, while Alan Maurer and Adam Deane advised on study design. Joyce James analyzed the pharmacokinetic data. All authors had access to the study data and reviewed and approved the final manuscript.

Acknowledgements

The authors would like to thank Paul Wischmeyer for providing patient samples for AAGP analysis and Andrew Day for statistical advice.

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Tables

Table 1. Effects of ulimorelin on gastric emptying in healthy volunteers, expressed as change from predose baseline, over the dose range of 80 to 600 µg*kg^{-1} Q8H on Days 1, 4 and 6.

Dose Group	Mean % Change in GE from Baseline (SD)								
	Day 1			Day 4			Day 6		
	N	Δt_{25}	Δt_{50}	N	Δt_{25}	Δt_{50}	N	Δt_{25}	Δt_{50}
Placebo	11	-12 (52)	-2.5 (19)	10	-14 (43)	-9 (14)	3^	7 (14)	-5 (21)
80 µg*kg^{-1}	9	37 (37)*†	16 (14)*†	8	14 (37)	16 (17)†	ND	ND	ND
150 µg*kg^{-1}	8	45 (23)*†	34 (14)*†	6	25 (52)	23 (8)*†	ND	ND	ND
300 µg*kg^{-1}	8	54 (27)*†	46 (14)*†	7	31 (25)†	27 (24) *†	ND	ND	ND
600 µg*kg^{-1}	9	45 (25)*†	44 (14)*†	8	22 (22)*†	25 (17)*†	8	36 (18)*†	28 (8)*†

Δt_{25}: percent change in time to 25% emptying, Δt_{50}: percent change in time to 50% emptying. Positive numbers indicate improvement, and negative numbers indicate prolongation of emptying. Baseline is Day minus 1. ^Day 6 in follow-on study only, in which 12 subjects were randomized 3:1 ulimorelin:plascebo. * $p \leq 0.05$ compared to Baseline (paired t-test), † $p \leq 0.05$ compared to placebo (t-test).

Table 2. Effects of ulimorelin 600 µg*kg^{-1} Q8H over 48 hours on colonic transit in healthy volunteers.

Endpoint	Placebo N=8 Mean (SD)	Ulimorelin 600 µg*kg^{-1} N=15 Mean (SD)	p-value
Colonic geometric mean center at 24 h	3.24 (1.02)	2.86 (0.78)	0.3258
Colonic geometric mean center at 8 h	1.42 (0.68)	1.28 (0.32)	0.0507
Colonic geometric mean center at 48 h	5.37 (1.19)	4.81 (1.21)	0.2974
Colonic filling at 6 hours (%)	50.13 (37.27)	23.53 (24.50)	0.0512
$t_{1/2}$ of ascending colon emptying (h)	10.53 (4.74)	10.76 (4.95)	0.9135
Percent retained in the colon at 8 h	90.94 (9.87)	89.93 (6.42)	0.7686
Percent retained in the colon at 24 h	90.04 (17.66)	96.40 (8.81)	0.2572
Percent retained in the colon at 48 h	64.31 (38.28)	77.38 (27.66)	0.3556

$t_{1/2}$: half-life

Figure Legends

Figure 1. Exposure-response modeling (E_{max}) of ulimorelin free C_{max} versus the improvement in the time to 50% liquid gastric emptying (Δt_{50}) on Days 1 and 4 in healthy volunteers.

Figure 2. AAGP distribution in patients and healthy volunteers.

Figure 1

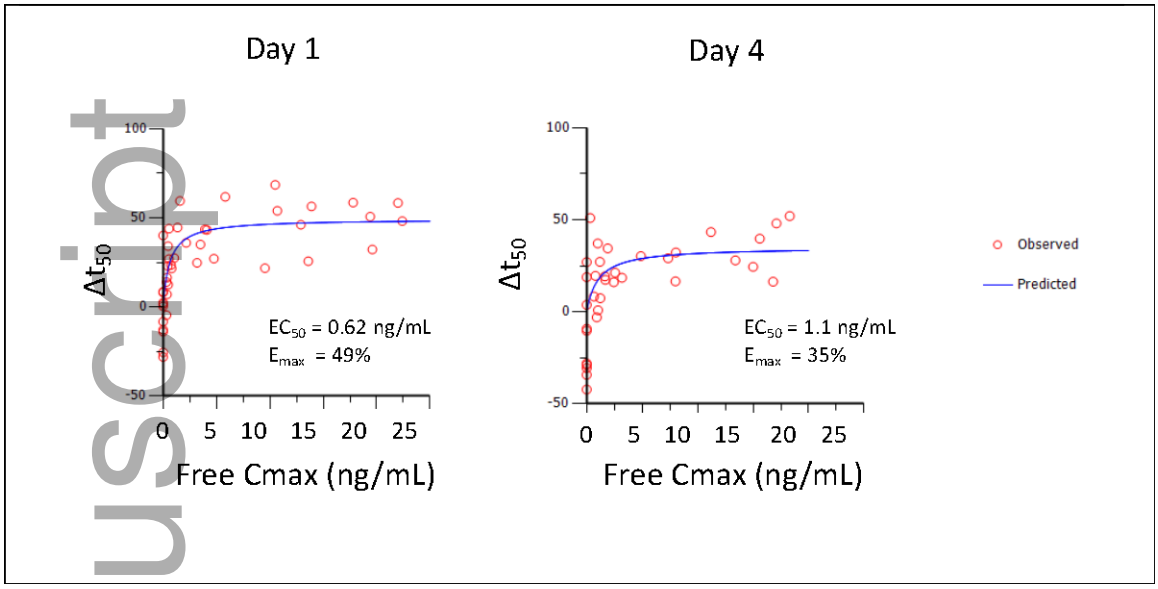

Figure 2