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Relamorelin: A Novel Gastrocolokinetic Synthetic Ghrelin Agonist

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Abstract

Synthetic ghrelin agonists, predominantly small molecules, are being developed as prokinetic agents that may prove useful in the treatment of gastrointestinal motility disorders. Relamorelin (RM-131) is a pentapeptide synthetic ghrelin analog that activates the growth hormone secretagogue (GHS)-1a (also called the ghrelin) receptor with approximately 6-fold greater potency than natural ghrelin. The ability of relamorelin to stimulate growth hormone (GH) release is comparable to that of native ghrelin. Relamorelin has enhanced efficacy and plasma stability compared to native ghrelin. In this review, we discuss the pharmacokinetics, pharmacodynamics and potential indications for relamorelin. Relamorelin is administered subcutaneously, dosed daily or twice daily. Relamorelin is being studied for the treatment of patients with gastrointestinal motility disorders. Phase IIA pharmacodynamic studies have demonstrated acceleration of gastric emptying in patients with type 1 diabetes mellitus (T1DM) and type 2 DM (T2DM) and upper gastrointestinal symptoms. In a phase IIA study in patients with diabetic gastroparesis, relamorelin accelerated gastric emptying and significantly improved vomiting frequency compared to placebo and improved other symptoms of gastroparesis in a pre-specified subgroup of patients with vomiting at baseline. In patients with chronic idiopathic constipation with defined transit profile at baseline, relamorelin relieved constipation and accelerated colonic transit compared to placebo. These characteristics suggest that this new ghrelin analog shows great promise to relieve patients with upper or lower gastrointestinal motility disorders.

Keywords

RM-131; gastroparesis; constipation

BACKGROUND

Currently Approved Medications for GI Motility Disorders

Disorders of gastrointestinal (GI) motility such as gastroparesis, post-operative ileus (POI), and chronic constipation are associated with significant morbidity. Development of effective

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pharmacologic therapies for such disorders is desirable, particularly for gastroparesis which represents a significant unmet medical need.

The only currently marketed medication for gastroparesis in the United States is metoclopramide (1), and its use is limited by a black box warning from the Food and Drug Administration (FDA) recommending use for less than three months for a chronic disease that may last several years. In other countries, domperidone is approved; its efficacy appears similar to that of metoclopramide (2) and, until relatively recently, it was considered safer than metoclopramide. However, a recent communication from the European Medicine Agency alerted prescribers and the public to the potential cardiac adverse effects (including prolongation of the QTc interval and cardiac arrhythmias) and, even, mortality (www.ema.europa.eu, accessed May 30, 2014) (3).

There is only one drug approved for POI; that is alvimopan which is administered at a dose of 12mg, 30 minutes to 5 hours prior to surgery, followed by 12mg twice daily, beginning the day after surgery until the patient is discharged from hospital, for a maximum of 7 days. However, it also has a black box warning because of the potential risk of myocardial infarction (www.fda.gov, accessed May 30, 2014) (4). Alvimopan is available only through the Entereg Access Support and Education (E.A.S.E.) Program that restricts use to enrolled hospitals. This program was required by the FDA because of the potential risk of myocardial infarction with long-term use of alvimopan, 0.5mg b.i.d, and duration of at least one month.

There are several medications available for the treatment of chronic idiopathic constipation (5) including medications available over the counter that accelerate colonic transit such as bisacodyl (6) and prescribed drugs such as the chloride secretagogues, linaclotide and lubiprostone (in the United States), and the 5-HT₄ receptor agonist, prucalopride (in virtually all countries other than the United States). None of these medications has been approved for the treatment of constipation in patients with neurological diseases such as paraplegia or Parkinsonism or in more severe forms of idiopathic constipation such as slow transit constipation or colonic inertia.

GHRELIN

Ghrelin is a 28-amino acid peptide which was first isolated from rat stomach. Ghrelin is identified in most gastrointestinal organs, predominantly in the stomach (Table 1 and Figure 1, upper panel). It is the natural ligand for the ghrelin or growth hormone secretagogue (GHS-1a receptor) and a potential target for treatment of clinical conditions associated with impaired gastric motility and energy balance (7). Administration of ghrelin promotes gastric motility in mice, rats, dogs and humans (7–9). However, the short half-life and plasma instability of native ghrelin limit its utility as a potential treatment for GI motility disorders. Synthetic ghrelin agonists, predominantly small molecules, are being developed as prokinetic agents that may prove useful in the treatment of GI motility disorders such as POI and gastroparesis (10). Actions and therapeutic pathways of ghrelin for upper gastrointestinal disorders have been reviewed elsewhere (11). In addition to ghrelin receptor expression in the central nervous system (CNS), there is growing evidence for ghrelin receptor expression throughout the gastrointestinal tract. Thus, whereas the protein

expression of ghrelin is far greater in the stomach than the small intestine and colon (12), the expression of ghrelin receptors is higher in the intestines than in the stomach, and these data are confirmed by the demonstration of ghrelin receptors in mucosa and myenteric plexus neurons of rodent colon (9) (Figure 1, lower panel and Figure 2). These observations support the potential for effects of ghrelin receptor agonists on colonic motor function. However, the reliability of the ghrelin receptor anti-GHSR1a antibodies has been recently questioned (13) and further studies are needed to characterize the presence and role of the ghrelin receptor in the colon.

THE GHRELIN AGONIST, RELAMORELIN

Chemistry

Relamorelin is a pentapeptide synthetic ghrelin analog with a molecular weight of 791 daltons (free base) and similar characteristics to native ghrelin. However, it has improved stability and longer plasma circulating half-life. The structure of relamorelin is not in the public domain; the holder of rights to relamorelin, Rhythm Pharmaceuticals has revealed that it is a synthetic pentapeptide.

Preclinical Pharmacology

In vitro—Relamorelin binds to the growth hormone secretagogue (GHS)-1a (or ghrelin) receptor with approximately 3-fold greater potency than natural ghrelin (K_i of relamorelin 0.42 ± 0.06 nM; K_i of h-ghrelin 1.22 ± 0.17 nM) studied in CHO-K1 cells, expressing the human recombinant GHS1a receptor. Relamorelin is also ~ 6-fold more potent than human ghrelin (h-ghrelin) in activating the ghrelin receptor and inducing downstream intracellular mediators, specifically intracellular calcium (EC_{50} of relamorelin 0.71 ± 0.09 nM; EC_{50} of h-ghrelin 4.2 ± 1.2 nM) determined in CHO-K1 cells, expressing the hGHS1a (14).

In vivo studies in animal models

Growth hormone release: The ability of relamorelin to stimulate growth hormone (GH) release in conscious freely moving rats is comparable to that of native ghrelin, with average peak GH concentrations of ~3000 ng/mL in response to i.v. administration of relamorelin and h-ghrelin at the same dose of 50 nmol/kg (data on file, Rhythm Pharmaceuticals). Relamorelin effects were noted in isolated smooth muscle from the human stomach and in circular smooth muscle from mouse and human colon. Hence, while GHSR1a has not been molecularly identified in the GI tract (15, 16), there is pharmacological evidence for expression of the GHSR1a in the GI tract (17).

In vivo studies in rats and dogs confirmed the *in vitro* studies that relamorelin increases GH levels similar to native h-ghrelin, while repeated dosing attenuated the effect on GH release (18). This attenuation is a potential advantage in long-term administration of the agent, as it would be expected to reduce any risks associated with chronically elevated GH levels.

Postoperative ileus: Relamorelin reversed laparotomy-induced POI in rats and was 15-fold more potent than native ghrelin. In normal Sprague-Dawley rats with more severe ileus induced by the combined effects of laparotomy and morphine administration, relamorelin

was potentially up to 300-fold more potent than natural ghrelin in reversing POI, and significantly reversed the delay in intestinal transit time (18).

Morphine-induced gastroparesis and small intestine ileus: Relamorelin reversed morphine-induced gastroparesis in normal Sprague-Dawley rats. Relamorelin was about 100-fold more potent than h-ghrelin in the morphine-induced delayed gastric emptying (Figure 3) (18).

Food intake and weight gain: Relamorelin increased food intake and induced weight gain in normal C57Bl6/J mice and Sprague-Dawley rats in a dose-dependent manner, and it induced a balanced increase in both fat and lean mass (19, 20). In multiple independent studies in normal rats, relamorelin increased body weight significantly beyond levels achieved with native ghrelin (20). Additionally, relamorelin increased food intake and body weight in animal models of cachexia (21–23), and similarly in Beagle dogs, it induced a significant, dose-dependent increase of body weight and food consumption, with no compromise in glycemic control (data on file, Rhythm Pharmaceuticals).

Anti-inflammatory activity: Relamorelin inhibited endotoxin-induced tumor necrosis factor (TNF)- α release *in vivo* as well as TNF- α -induced lipolysis *in vitro*. In cachexia models, relamorelin reduced the elevated levels of interleukin-1, interleukin-6, interleukin-12, and TNF- α to control levels (21). In a mouse model of inflammatory bowel disease induced by intra-rectal administration of trinitrobenzene sulfonic acid (TNBS), relamorelin improved survival, reduced signs of macroscopic colonic inflammation, and reversed weight loss when compared to controls (18).

Comparison of relamorelin to ghrelin and other ghrelin agonists: Relamorelin was compared to other investigational ghrelin agonists, anamorelin, ibutamoren and ipamorelin, in the rat POI plus morphine model. The results showed relamorelin had a great effect on gastric emptying when compared to each of the other agents. In this assay, relamorelin was ~100-fold more potent than native ghrelin. In the rat morphine model of gastric dysmotility, relamorelin was 600- to 1800-fold more potent than TZP-101 and TZP-102, two other ghrelin receptor agonists (18).

Preclinical Toxicology

Toxicological profiles of relamorelin have been evaluated in studies using repeated dose subcutaneous (s.c.) injection of up to 13 weeks in rats and monkeys. Overall, the 13-week repeat-dose toxicological studies provide very large safety margins for clinical investigation. There are margins of >100-fold based on dose (mg/m²; assuming a 70kg human), > 330-fold based on C_{max} exposures, and >760-fold based on AUC (data on file, Rhythm Pharmaceuticals).

Pharmacokinetics and Metabolism

Relamorelin is administered subcutaneously and is considered to be metabolically stable, as no metabolites have been detected *in vitro* in any of the species tested. The mean percentage

binding *in vitro* of ^{14}C -relamorelin to serum proteins ranged from 83% to 96% with no substantial differences in rat, dog, or human sera.

In humans, across all dosing groups studied as well as after single or multiple administrations for 10 days, the median time to peak plasma concentration ($T_{\max}$) was 0.74 hour (minimum and maximum 0.27 and 1.02 hour, respectively). The relamorelin $C_{\max}$ increased proportionately with dose across all doses, with $C_{\max}$ of $74.8 \pm 15.3 \text{ ng/mL}$ at the highest dose (2400 μg) tested. The plasma relamorelin AUC was proportional to dose across all doses. In a single-ascending dose study, relamorelin was rapidly absorbed and appeared to exhibit two-compartment pharmacokinetics. Across all dose groups, the average relamorelin half-life was ~4.5 hours in the single ascending dose study, but measurements were limited at later time points; a relatively long mean terminal half-life of ~19 hours was observed at the 2400 μg dose, which was the only dose 24 hours post-administration that was associated with blood levels above the lower limit of quantification (LLOQ; 0.1 ng/mL). There was no apparent accumulation of relamorelin after repeated administration for 10 days.

The pharmacokinetics profile of RM-131 was similar after morning and evening administration of 5 and 10 μg , b.i.d, with no detectable drug levels observed in the morning following evening administration of 10 μg .

The pharmacokinetics of relamorelin appears similar in healthy volunteers and patients with diabetes mellitus. Across all dose groups, no metabolism has been detected in human microsomes and hepatocytes, and approximately 8% of the dose was excreted in urine.

Safety and Tolerability in Humans

Relamorelin has been evaluated in a single-ascending dose clinical study, over a dose range of 3 to 2400 μg relamorelin or placebo in 36 healthy male volunteers, of whom 27 received relamorelin and 9 received placebo. Assessments of laboratory parameters, ECGs, adverse event occurrences, vital signs and injection site evaluations suggest that relamorelin was well tolerated at all dose levels. There were no study discontinuations due to adverse events, and there were no serious adverse events or deaths.

Relamorelin has also been evaluated in a 14-day, multiple-ascending dose study in 40 healthy male and female adults, of whom 32 received relamorelin and 8 received placebo. Each participant received a constant total daily dose of relamorelin (subcutaneous doses of 10, 20, 80, or 300 μg). Relamorelin was generally well tolerated at doses up to 300 μg per day by evaluation of laboratory parameters, ECGs, adverse event occurrences, vital signs and injection sites.

To date, over 240 persons have been exposed to relamorelin at doses ranging from 3 to 2400 μg in seven clinical studies. Of these, 59 were healthy volunteers; 27 received a single relamorelin dose and 32 received repeated doses over 14 days. In addition, approximately 210 patients with type 1 or type 2 diabetes mellitus and gastroparesis, and 24 with functional constipation have received multiple doses of relamorelin. In these different groups, relamorelin was consistently well-tolerated.

Drug Interactions

The IC₅₀s for inhibition of CYP3A4/5, CYP2A6, CYP2C8, CYP2C19, and CYP2D6 were all >15μM. Therefore, the potential for relamorelin to inhibit the metabolism of concomitantly administered drugs is weak, considering that the highest dose administered to humans (300μg/day, Study RM-131-002) only achieved a mean C_{max} of 13ng/mL (approximately 0.02μM).

Pharmacodynamic Studies in Humans

Pharmacodynamic evaluations of relamorelin demonstrated evidence for robust biological activity, including increased GH levels and acceleration of gastric emptying by gastric emptying breath test following administration of RM-131. In a double-blind, placebo-controlled, single-ascending dose study, doses of 3 to 2400μg relamorelin were administered by subcutaneous injection once daily in nine separate cohorts of four healthy volunteers (3 active/1 placebo) and gastric emptying was assessed by breath test. In addition, relamorelin was also evaluated in a multiple-ascending dose study at 10 to 300μg subcutaneously once or twice daily for 14 days in four separate cohorts (8 active/2 placebo). Accelerated gastric emptying was demonstrated after single and multiple doses of 10μg relamorelin (35 to 55% change from baseline; p<0.05). Gastric emptying results on days 1 and 7 were similar, with a comparable change in gastric emptying following daily doses of 10, 20, 80 and 300μg relamorelin (24)].

EFFECTS OF RELAMORELIN ON GASTRIC EMPTYING IN GASTROPARESIS

Phase IB Studies in Patients with Diabetes Mellitus and Prior Documentation of Delayed Gastric Emptying

Results from two small, phase IB, placebo-controlled, single-dose (100μg subcutaneously), 2-period, cross-over studies in patients with type 2 (25) and type 1 (26) diabetes mellitus and prior documentation of delayed gastric emptying indicated that relamorelin was generally well tolerated. Relamorelin significantly decreased gastric retention of solids at 1 and 2 hours; the solid gastric emptying T_{1/2} was faster in patients with type 1 diabetes mellitus, with a median difference of -33.9 minutes (equivalent to -54.7% decrease) (26). Analysis of primary efficacy data showed relamorelin was associated with significant acceleration of T_{1/2} of gastric emptying of solids assessed by scintigraphy (Figure 4) (25). In patients with type II diabetes mellitus, there were also significant differences in the symptoms of gastroparesis during treatment with relamorelin compared to placebo. Thus, the total gastroparesis cardinal symptom index-daily diary (GCSI-DD) scores (27, 28) were 0.79 (IQR, 0.75, 2.08) on placebo and 0.17 (IQR, 0.00, 0.67; P=0.026) on relamorelin; similarly, a composite of nausea, vomiting, fullness and epigastric pain scores were lower on relamorelin (P=0.041) compared to placebo (26).

Phase 2 Clinical Trial of RM-131 in Diabetic Gastroparesis

A randomized, double-blind, placebo-controlled, adaptive design, parallel-group, 28-day study was conducted in patients with diabetic gastroparesis. The study design consisted of

an initial 1-week, single-blind, placebo run-in, followed by randomization in double-blind fashion to s.c. placebo b.i.d. or relamorelin, 10µg s.c. b.i.d. (before breakfast and evening meal), or relamorelin, 10µg s.c. q.d. (placebo before breakfast and relamorelin before evening meal). For eligibility, all eligible patients had: baseline gastric emptying $T_{1/2} > 79$ minutes by ^{13}C spirulina breath test, Gastroparesis Cardinal Symptom Index (GCSI) score ≥ 2.6 , and a history of nausea and/or vomiting ≥ 1 /week during the 2 weeks prior to screening. Compared to baseline, relamorelin, 10µg b.i.d., resulted in significant acceleration of gastric emptying ($p < 0.03$). There were also significant improvements in vomiting endpoints on relamorelin treatment compared to placebo; these effects were most impressive in the ~60% patients who had vomiting during the baseline period. In the subgroup of patients with vomiting at baseline, other symptoms of gastroparesis were also significantly improved. Overall, there were no safety concerns, few adverse events, and no significant effects on body weight (29).

EFFECTS OF RELAMORELIN ON COLONIC TRANSIT IN CHRONIC CONSTIPATION

Ghrelin receptors are identified throughout the gastrointestinal tract, including the colon (12); as indicated above, the expression of ghrelin receptors in rodent intestine is actually higher in small and large intestine than in the stomach. Relamorelin hyperpolarized resting membrane potential of human colon circular smooth muscle cells *in vitro* (17), suggesting that it has also functional effects on colonic motility. In an *in vivo* model in conscious mice, with a miniaturized pressure transducer catheter introduced into the colon ~2.5cm proximal to the anus, i.p. relamorelin reduced phasic contractile activity in the colon. Others have also shown that i.t. ghrelin induced strong propulsive contractions in the distal colon. These effects are thought to be mainly mediated by activating the defecation center in the L1-S3 lumbo-sacral region (30, 31). Thus, the site of stimulation of colonic transit appears to be peripheral. Shimizu et al. showed that acyl-ghrelin stimulated rat colonic contractility when given intravenously or intrathecally, but not when given into the 4th ventricle. Additionally, similar delivery methods had no effect on small bowel motility, which would be a manifestation of vagal stimulation in the brainstem. They concluded that ghrelin activates pelvic nerves at the sacral defecation center, innervating the enteric neurons in the distal colon (31). On the contrary, other authors have shown that ghrelin has no effect on contractility in the rat stomach, distal colon, human ascending and human sigmoid colon (32, 33). It has been suggested, from rat studies, that GHSR1a agonists affect colonic motility through actions in the CNS (34). However, in preclinical studies in rats, relamorelin had a low penetration in the CNS, showing that availability of relamorelin in the brain of rats is 0.015% at 5mg/kg (data on file, Rhythm Pharmaceuticals). It is therefore possible that relamorelin acts at a different receptor to those receptors (including GHSR1a) that are activated by ghrelin. Hence, further studies are needed to understand the mechanism and site of action of relamorelin.

A preliminary report documented the propulsive effects of relamorelin in humans with chronic constipation. In a single-center, randomized, double-blind, placebo-controlled, parallel-group study of a single daily dose of 100µg, s.c. relamorelin for 14 days, there was

significant improvement in bowel functions, relieving constipation and significant acceleration of gastric emptying $T_{1/2}$, colonic filling at 6 hours (surrogate for small bowel transit), and overall colonic transit at 32 and 48 hours. Hunger or increased appetite were reported more frequently in patients who received relamorelin compared to placebo ($p=0.012$) (35).

Safety Profile in Humans

The pilot studies in patients with type 1 and type 2 diabetes mellitus indicated that 100 μ g relamorelin was well tolerated with no serious adverse events, deaths, or adverse events leading to withdrawal. The most common adverse events reported in these single-dose, pilot studies were hyperhidrosis and dizziness; fatigue and abdominal pain/cramping; and decreased blood pressure, hunger, feeling cold, and muscular weakness (25).

In the larger study in over 200 patients with diabetic gastroparesis, the safety and tolerability profiles of relamorelin were generally good, the number of treatment-emergent adverse effects were low, and there were no imbalances or concerns identified by physical examinations, ECGs, vital signs, and safety laboratory tests including liver function tests. In addition, there were no clinically relevant injection site reactions or changes in weight or fasting blood glucose (29). In a crossover, placebo-controlled study in 6 healthy volunteers, a ghrelin agonist produced a decrease in insulin sensitivity (which might theoretically represent a diabetogenic effect) and stimulated lipolysis (36).

The long-term adverse effects and safety of relamorelin are under evaluation. A clinical review of ghrelin administration for multiple indications other than gastroparesis or constipation showed that the main potential side effect of ghrelin is increased appetite (37). Ghrelin plays a key role in stimulation of appetite in healthy individuals by stimulating the appetite centers in the hypothalamus, including the agouti-related peptide and neuropeptide Y receptors in the arcuate nucleus of the hypothalamus (reviewed in (38)). Additionally, ghrelin stimulates the secretion of orexins, growth hormone, insulin-growth factor 1, and insulin, which have orexigenic properties (11). Conversely, increased appetite may be a desirable effect in certain populations such as cachexia due to cancer (39), chronic obstructive pulmonary disease (40) or advanced heart disease (41). There is a theoretical risk that ghrelin may be involved in autocrine and paracrine processes resulting in cancer progression, including cell proliferation, cell migration and apoptosis (42). Currently, it is unclear whether ghrelin induces cell proliferation or inhibits cell proliferation, apoptosis and angiogenesis (43). It is also conceivable that the anti-inflammatory properties of ghrelin may have a protective role for some cancers (44). In the published human studies to date, there is no association of exogenous ghrelin or ghrelin agonist administration with cancer progression, and this includes patients who received these medications for cancer-induced cachexia (37). The potential effects attributable to stimulation of ghrelin receptors with relamorelin require more thorough evaluation with long-term administration.

CONCLUSIONS

Relamorelin is a pentapeptide synthetic ghrelin analog that is well tolerated and accelerates gastric emptying, small bowel transit and colonic transit in humans with gastroparesis and

chronic constipation. These promising data and the safety profile of relamorelin suggest that further studies and development of this medication are warranted. In addition, given the pharmacodynamics data observed to date, relamorelin should be explored for the treatment of organic, neurological constipation, in addition to its effects in functional or chronic idiopathic constipation.

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Figure 1.

Ghrelin and ghrelin receptor expression in gastrointestinal tract. Representative electrophoretic analysis patterns of the RT-PCR products of ghrelin and GHS-R mRNAs in rats. Upper panel: Ghrelin transcripts in the stomach and small and large intestines. Lower panel: GHS-R transcripts in those same organs. Reproduced with permission from Date, Kojima, et al. 2000.

Figure 2.

GHS-R-immunofluorescence (green) in transverse frozen sections of the rat distal colon observed in (A) neuronal cell bodies and nerve fibres of the myenteric plexus, and (B) cells scattered in the mucosa, exhibiting an entero-endocrine and/or mast cell-like distribution, size and density. Reproduced with permission from Dass NB, et al. Neuroscience 2003.

Figure 3.

Percent of gastric emptying (mean±SEM) at baseline and in different rat gastric dysmotility models: POI, POI plus morphine, or morphine-only model (Panel A), and the morphine-only model of delayed gastric emptying (Panels B and C). Animals received the phenol red-marked meal by esophageal gavage. Vehicle is 0.9% NaCl. Data show mean ± standard error of the mean (SEM). Percentage of phenol-red gastric emptying rate (% GE) determined. Groups of rats were treated each as indicated and dosed with RM-131 or h-ghrelin. Reproduced with permission from Van der Ploeg, Laken, et al. 2014.

Figure 4. Effects of RM-131 in type 2 diabetic gastroparesis patients with delayed gastric emptying. Reproduced with permission from Shin, Camilleri, et al. Clin Gastroenterol Hepatol 2013.

Table 1
Tissue Content of Immunoreactive Ghrelin

(reproduced from Date Y, et al. Endocrinology 2000)

Tissue	RIA for C-terminus (pmol/g wet wt)	RIA for N-terminus (pmol/g wet wt)
Stomach		
Fundus	4633.5 ± 440.1	1845.6 ± 290.0
Pylorus	120.7 ± 19.4	63.6 ± 21.1
Duodenum	262.5 ± 13.3	50.9 ± 7.2
Jejunum	102.3 ± 13.2	44.6 ± 8.4
Ileum	27.7 ± 1.1	1.5 ± 0.1
Colon	73.4 ± 19.2	11.7 ± 4.2

Values are the mean ± SEM (n = 6).