Efficacy of Ipamorelin, a Novel Ghrelin Mimetic, in a Rodent Model of Postoperative Ileus

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ABSTRACT

Ghrelin and ghrelin mimetics stimulate appetite and enhance gastric motility. The present study investigates whether ipamorelin, a selective growth hormone secretagogue and agonist of the ghrelin receptor, would accelerate gastrointestinal transit and ameliorate the symptoms in a rodent model of postoperative ileus (POI). Fasted male rats were subjected to laparotomy and intestinal manipulation. At the end of surgery, a dye marker was infused in the proximal colon to evaluate postsurgical colonic transit time, which was the time to the first bowel movement. In addition, fecal pellet output, food intake, and body weight were monitored regularly for 48 h. Ipamorelin (0.01–1 mg/kg), growth hormone-releasing peptide (GHRP)-6 (20 µg/kg), or vehicle (saline) were administered via intravenous bolus infusion after a single dosing or a 2-day repetitive dosing regimen (four doses a day at 3-h intervals). Compared with the vehicle, a single dose of ipamorelin (1 mg/kg) or GHRP-6 (20 µg/kg) decreased the time to the first bowel movement but had no effect on cumulative fecal output, food intake, or body weight gain measured 48 h after the surgery. In contrast, repetitive dosing of ipamorelin (0.1 or 1 mg/kg) significantly increased the cumulative fecal pellet output, food intake, and body weight gain. The results suggest that postsurgical intravenous infusions of ipamorelin may ameliorate the symptoms in patients with POI.

Postoperative ileus (POI) is a gastrointestinal (GI) dysfunction that develops as a consequence of abdominal surgery or other major surgical procedures. Abdominal distention, nausea, vomiting, anorexia, and an inability to pass stool are the main symptoms of POI. Although normal GI motility returns spontaneously within several days in most patients, POI is the main cause for prolonged hospitalization and often leads to serious complications in others (Senagore, 2007). The mechanisms causing POI are complex, involving immune and neuronal reactions to the surgery that result in delayed gastric emptying and impaired propulsive motility of the bowel (Bauer and Boeckxstaens, 2004). In addition, opioid drugs used for pain management contribute significantly to the delay in GI transit.

POI is a generalized event involving the entire GI tract and not restricted to the region directly subjected to manipulation. Manipulation of the small intestine in the rat was found to inhibit muscle contractility and evoke inflammatory responses in the stomach and the colon (Kalff et al., 1998; Schwarz et al., 2004), whereas manipulation of the cecum delayed gastric emptying (Martínez et al., 1999). Moreover, the time course of functional recovery varies in different anatomical regions of the GI tract. In humans, small intestinal motility recovers within several hours, whereas the recovery of gastric function takes 1 to 2 days, and colonic propulsive motility requires at least 2 to 3 days for recovery (Condon et al., 1986). There are currently no pharmacological agents available to normalize gut motility, and the pharmacological strategies proposed to accelerate recovery from POI are based on the effects of commonly used prokinetic drugs and/or selective periphery-restricted opioid receptor antagonists (for review, see Greenwood-Van Meerveld, 2007). The treatment of POI is limited to supportive measures to correct nutrition and fluid deficits and promote GI function by early feeding, ambulation, or selective use of nasogastric decompression to alleviate the symptoms. Because anorexia is a major complication during the postoperative period, the capability of a novel agent to promote food intake could also be
of benefit. Most recently, a new class of agents that mimic the activity of endogenous ghrelin by exhibiting both prokinetic and orexigenic effects has been proposed for the treatment of POI. TZP-101 (Hoveyda et al., 2006), a small molecule with potent binding affinity and full agonist activity at the human recombinant ghrelin (GRLN) receptor, is now in clinical development (Lasseter et al., 2008).

The GRLN receptor has been previously identified as an orphan receptor referred to as the growth hormone-releasing peptide (GHRP) receptor or growth hormone secretagogue receptor, because its activation by synthetic small peptide compounds resulted in the release of growth hormone from the pituitary (Davenport et al., 2005). More than 10 years ago, ipamorelin was characterized as the first selective GHRP receptor agonist showing no significant effect on plasma ACTH and cortisol levels (Raun et al., 1998). Later, a 28-amino acid peptide isolated from rat stomach was discovered as the endogenous ligand of the orphan receptor and called ghrelin (Kojima et al., 1999). Endogenous ghrelin is a multifunctional hormone involved in the regulation of appetite, feeding behavior, and GI motility. Because regulation of food intake (Ueno et al., 2005) and stimulation of gastric motility and secretion (Masuda et al., 2000; Fujino et al., 2003) are among the most important biological effects of ghrelin, treatment with ghrelin mimetics or ghrelin could be of benefit to the patient after surgery. Preclinical studies in rodent models of POI have shown that both ghrelin and small molecule synthetic agonists of the GRLN receptor accelerate gastric emptying (Trudel et al., 2002; Poitras et al., 2005; Venkova et al., 2007). The goal of this study was to investigate whether ipamorelin, a synthetic peptidomimetic that selectively stimulates the ghrelin receptor and causes growth hormone release (Raun et al., 1998), would accelerate GI transit in a rat model of POI. The results support the concept that repetitive intravenous infusions of the GRLN receptor agonist ipamorelin could improve GI motility and support the overall recovery after abdominal surgery. Clinical development of ipamorelin for the treatment of POI is underway.

Materials and Methods

Animals. Adult male Sprague-Dawley rats were obtained from Charles River Laboratories, Inc. (Wilmington, MA) at an initial body weight of 250 to 270 g. An acclimation period of at least 1 week was allowed before the experiments. All rats were single-house with free access to food and water at 21 to 23°C and a 12-h light/dark cycle. The animals were purchased with indwelling catheters implanted in the right jugular vein for administration of drugs or vehicle. The catheters were maintained patent by gently flushing with 0.2 to 0.3 ml of heparinized saline every 3 to 4 days. An additional group of control rats, not subjected to surgery and drug or vehicle treatment, was purchased with an indwelling catheter implanted into the proximal colon (1–2 cm from the cecum) used for infusion of a dye marker measuring colonic transit. The colonic catheters were flushed with 0.2 ml of saline every 3 to 4 days. The experimental protocol was approved by the Animal Studies Subcommittee, Research and Development Committee and the Institutional Animal Care and Use Committee at the Oklahoma City Veterans Administration Medical Center and University of Oklahoma Health Sciences Center.

Abdominal Surgery. POI was induced by a surgical procedure described as “running of the bowel” (Kalf et al., 1998). Rats were anesthetized with isoflurane (2–3%) inhalation, the abdomen was shaved, the area was treated with alcohol and then a povidone-iodine topical antiseptic for disinfection, and a midline incision was made to expose the viscera. The small intestine and the cecum were exteriorized, inspected for 5 min using cotton applicators soaked in sterile saline, and then covered with gauze soaked in saline for an additional 10 min. At the end of the surgery, the small intestine and the cecum were placed back into the abdominal cavity, and the incision was closed with running silk sutures. The surgical procedure lasted 25 to 30 min and was always performed at 6:00 to 8:00 AM.

Colonic Transit Time. Before the experiments all rats were fasted for 20 to 22 h with free access to water. To evaluate colonic transit, 200 l of a nonabsorbable dye marker (trypan blue in saline) was injected into the proximal colon at the end of the surgical procedure. The abdomen was closed, and the rats were placed in clean home cages supplied with preweighed food (Purina rat chow) and water. Within a period of 5 to 10 min, the rats regained consciousness and started ambulating in the home cage. Colonic transit time, which was also the time to the first bowel movement, was evaluated as the period between the end of surgery and the appearance of dye in the fecal pellet. A control rat, not subjected to surgery and drug or vehicle treatment, was studied on each experimental day together with the rats with POI. The control rats were equipped with colonic catheters used to infused the dye marker into the colon after a 20- to 22-h fasting.

Cumulative Fecal Output, Food Intake, and Body Weight. In the rats treated with a single dose of ipamorelin fecal pellets were counted and weighed at 3-h intervals during the first 12 h after surgery and then at 24 and 48 h postsurgery. The cumulative fecal output was evaluated by adding the number of pellets at 12, 24, and 48 h postsurgery. Food intake was recorded at the same time points according to the experimental design and was normalized as grams per 100 g of body weight. In the rats subjected to multiple dosing of ipamorelin, fecal output and food intake were measured after each administration of ipamorelin between 0 and 12 and 24 and 36 h after the surgery. The data from all time points were subjected to regression analysis to evaluate the rate of recovery of fecal output and food intake in the rats receiving ipamorelin or vehicle treatment on the 1st and 2nd days after the surgery. The cumulative food intake was calculated for 48 h postsurgery. Body weight was measured daily at 6:00 to 8:00 AM after fasting the animals, on the day of experiment before the surgery and at 24 and 48 h postsurgery. Changes in body weight are expressed as body weight gain compared with the weight of the fasted animal taken before the surgery.

Test and Control Compounds. The test compound, ipamorelin, is a pentapeptide (Alb-His-d-2-Nal-d-Phe-Lys-NH2) (Raun et al., 1998). Ipamorelin-free base (Albany Molecular Research, Inc., Albany, NY) used in the experiments was provided by Sapphire Therapeutics, Inc. (Bridgewater, NJ). Because the solubility of ipamorelin is very low, 2 M equivalents of glacial acetic acid are required to produce a soluble diacette salt. Stock solutions of 0.5 mg/ml were prepared daily in sterile saline plus glacial acetic acid (0.1 l/ml) to bring ipamorelin into solution, pH 3 to 4. The solution was titrated with NaOH to pH 7.0 to 7.2. Additional dilutions were made in saline. Sterile saline was used in the vehicle control experiments. The positive control GHRP-6 (His-d-Trp-Ala-Trp-d-Phe-Lys-NH2) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in sterile saline.

Experimental Design. The animals received ipamorelin or vehicle treatment during the light phase of the light/dark cycle. Both the test and control compounds were administered as a bolus intravenous infusion via the jugular catheter at a volume of 0.2 ml/kg body weight. Two series of experiments employing a single or repetitive administration of ipamorelin were performed to evaluate the ability of ipamorelin to reduce the symptoms of POI. The growth hormone secretagogue GHRP-6 (20 l/kg) was used as a positive control because it has been reported to accelerate gastric emptying in mice at a dose range of 20 to 100 l/kg (De Winter et al., 2004). In the first series, ipamorelin or vehicle was administered as a single bolus intravenous infusion at the end of the surgery at doses of 0.1 to 1 mg/kg. Colonic transit time was evaluated by the appearance of the...
first marked fecal pellet. Cumulative fecal pellet output and food intake were measured at 3-h intervals during the first 12 h and at 12-h intervals until 48 h after surgery. In the second series, ipamorelin at doses of 0.01, 0.1, or 1 mg/kg or vehicle was administered according to a repetitive dosing regimen initiated at the end of surgery. Two series of four consecutive bolus infusions performed at 3-h intervals were administered between 8:00 AM and 5:00 PM on the 1st and 2nd postoperative days. Fecal pellet output and food intake were measured at 3-h intervals after each of the individual doses. The dosing regimen was based on the relatively short 2-h half-life of ipamorelin (Gobburu et al., 1999). All animals were weighed 1 day before the surgery, after a 20- to 22-h fasting before surgery and at 24 and 48 h postsurgery. Four surgeries were performed on each experimental day, and the doses of ipamorelin were given in a randomized manner. A vehicle control and a control rat (not subjected to surgery) were studied simultaneously with the rats receiving ipamorelin on each experimental day.

Data Analysis and Statistics. The data are expressed as the mean ± S.E.M. for each group. Differences between groups were assessed for statistical significance by Student’s t test and by one- or two-way ANOVA followed by Dunnett’s or Bonferroni’s test for multiple comparisons where appropriate. A level of p < 0.05 was considered significant. In addition, the effects of multiple dosing were evaluated using linear regression analysis of individual data. The statistical procedures were performed using Prism software (version 4; GraphPad Software Inc., San Diego, CA).

Results

The Rat Model of POI. To study the effect of surgery on GI function, we evaluated surgery-induced inhibition of the propulsive motility of the colon by measuring the colonic transit time to the first bowel movement after surgery and the cumulative fecal output for 48 h. Food intake and body weight were also measured. In the present study, abdominal surgery was found to delay colonic transit, causing a significant increase of the time to the first bowel movement registered by the appearance of a colored fecal pellet (10.4 ± 0.6 h in rats subjected to surgery versus 6.6 ± 0.6 h in control rats, p < 0.05, Student’s t test). The cumulative fecal pellet output was significantly decreased during the first 12 and 24 h after surgery (Fig. 1A). When placed in the home cage for observation, control rats and rats subjected to surgery ingested approximately the same amount of food during the first 0 to 3 h. However, at later periods of 12, 24, and 48 h, food intake was suppressed in the rats with POI (Fig. 1B). In accordance, the body weight gain in the rats with POI was significantly lower at 24 and 48 h in comparison with control rats (Fig. 1C).

Effects of a Single-Dose Treatment with Ipamorelin in Rats with POI. Experiments were performed in rats with POI to investigate the efficacy of a single-dose treatment with ipamorelin at doses of 0.1 or 1 mg/kg. Ipamorelin or the vehicle was administered via intravenous infusion upon completion of the surgery, and efficacy was evaluated during a 48-h recovery period by comparing the effects of ipamorelin with the effect of the vehicle. In addition, the effect of the GRLN receptor agonist GHRP-6 administered at a dose of 20 µg/kg was studied as a positive control. The results presented in Fig. 2 demonstrate that a single postsurgical dose of ipamorelin (1 mg/kg) or GHRP-6 significantly decreased colonic transit time shortening the time to the first bowel movement. However, neither ipamorelin nor GHRP-6 had any significant effect on cumulative fecal pellet output (Fig. 3A), food intake (Fig. 3B), or body weight gain (Fig. 3C), measured at 12, 24, and 48 h after surgery.

Effects of Repetitive Dosing of Ipamorelin in Rats with POI. In a separate series of experiments, we investi-
gated the dose-response effect of repetitive dosing of ipamorelin (0.01, 0.1, or 1 mg/kg i.v.) during the first 48 h postsurgery. The colonic transit time to the first bowel movement was significantly decreased by 0.1 or 1 mg/kg ipamorelin compared with the effect of the vehicle (Fig. 4). In addition, the effect of ipamorelin on cumulative fecal pellet output was associated with an increase in the number of fecal pellets. The number of fecal pellets produced during the 48-h postsurgical period was significantly higher at doses of 0.1 or 1 mg/kg (Fig. 5A). Regression analysis of the individual data for each treatment group demonstrated a linear increase of fecal output during treatment with ipamorelin (0.01–1 mg/kg) or vehicle (Fig. 5B). However, fecal output increased at a higher rate in the rats receiving ipamorelin at doses of 0.1 or 1 mg/kg compared with rats treated with vehicle (Table 1). The repetitive dosing of ipamorelin caused a similar increase in food intake (Fig. 6, A and B; Table 1). The rats receiving 1 mg/kg ipamorelin gained significantly more weight during the first 48 h after surgery compared with the rats treated with the vehicle (Fig. 7).

**Discussion**

The rat model of POI induced by manipulation of the bowel is commonly used to investigate the pathogenic mechanisms relevant to the postsurgical development of GI dysfunction in humans and to investigate the efficacy of new therapeutic treatments. In previous studies, rats with POI showed a significant delay in colonic transit times and a decrease in fecal pellet output and food intake compared with control animals (Fraser et al., 2009). Data presented in this study confirm that colonic transit, food intake, and body weight gain are suppressed during the first 48 h after abdominal surgery and provide new evidence that ipamorelin, a peptidomimetic acting as an agonist of the GRLN receptor (Raun et al., 2009), has an effect on these parameters.

**Fig. 3.** Single dose treatment with ipamorelin (0.1–1 mg/kg) or GHRP-6 (20 µg/kg) had no significant effect on the cumulative fecal pellet output (A), food intake (B), and body weight gain (C) in rats subjected to abdominal surgery. Data are mean ± S.E.M. from 9 to 12 rats. Differences were assessed for statistical significance by two-way ANOVA (p > 0.05).

**Fig. 4.** Colonic transit time, which was also the time to the first bowel movement after surgery, was decreased by ipamorelin (0.1–1 mg/kg). Data are mean ± S.E.M. from eight to nine rats per group. Statistical significance of the differences was evaluated by one-way ANOVA (p < 0.05) and Dunnett’s post hoc test for multiple comparisons with the vehicle: *, p < 0.05.

**Fig. 5.** A, effects of repetitive dosing of ipamorelin (0.01–1 mg/kg) on the cumulative number of fecal pellets produced during the 48-h period after surgery. Data are mean ± S.E.M. from eight to nine rats per group. Significance of the differences was evaluated by two-way ANOVA (p < 0.05) and Bonferroni’s post hoc test for multiple comparisons: *, p < 0.05 compared with vehicle. B, regression analysis of the individual data for each treatment group demonstrated that fecal output increased at a higher rate in the groups treated with 0.1 or 1 mg/kg ipamorelin. Graph points represent mean ± S.E.M. for each group.
 accelerates the recovery of colonic transit and stimulates food intake and body weight gain in rats with POI. The multifactorial effect of ipamorelin, including the increase in fecal pellet output, food intake, and body weight gain, was evident with a repetitive dosing regimen, whereas the effect of a single intravenous infusion was expressed only by the decrease in the time to first bowel movement. In clinical practice, drugs are rarely administered in a single dose to produce a desired effect; instead, repetitive dosing is used to maintain drug plasma concentrations producing a lasting result. The dosing regimen used in our study was based on the relatively short 2-h half-life of ipamorelin (Gobburu et al., 1999) to ensure that a plasma concentration of ipamorelin was maintained for a duration sufficient to achieve full functional activity.

Ipamorelin is a potent synthetic pentapeptide with distinct and specific growth hormone-releasing properties (Raun et al., 1998; Jiménez-Reina et al., 2002). The potency and efficacy of ipamorelin are similar to GHRP-6 (Raun et al., 1998); however, unlike GHRP-6, ipamorelin does not increase plasma levels of ACTH or cortisol. In vitro, ipamorelin was found to release growth hormone from primary rat pituitary cells, and this effect was antagonized by D-Lys3-GHRP-6, an antagonist of the GRLN receptor. In addition to its action as a growth hormone secretagogue, ipamorelin shows other activities known to occur via agonist of the GRLN receptor. Ipamorelin, administered subcutaneously twice a day in adult female rats, was found to increase the cumulative daily food intake and body weight during the first 72 h of treatment (Lall et al., 2001). This effect was independent from stimulation of growth hormone release and was most probably mediated by GRLN receptors on neurons in the arcuate nucleus of the hypothalamus, which release neuropeptide Y and/or agouti-related peptide regulating food intake and energy homeostasis (Cowley et al., 2003). The effect of ipamorelin seems to be similar to the effect of ghrelin, which was
found to activate hypothalamic neurons and stimulate food intake when injected into the periphery (Nakazato et al., 2001), despite the very low rate at which ghrelin passes the brain-blood barrier (Banks et al., 2002). Because the ability of peripheral ghrelin to stimulate food intake was inhibited by vagotomy, it has been suggested that activation of GLRN receptors on vagal nerve afferents may be important for the orexigenic effect of ipamorelin in rats (Date et al., 2002) and humans (le Roux et al., 2005). With regard to a possible direct interaction of ipamorelin with central GLRN receptors, one can speculate that like endogenous ghrelin, ipamorelin could penetrate the relatively permeable blood-brain barrier at the area postrema and reach GLRN receptors in neighboring structures, such as the arcuate nucleus of the hypothalamus and the nucleus of the tractus solitarius in the brainstem (Imai, 2001).

Another important aspect of the physiological role of endogenous ghrelin is the stimulation of gastric motility that has been documented and studied in detail in humans and rodents (for review, see Peeters, 2006). Ghrelin increases gastric emptying and induces fasted motor activity in fed animals and humans by modulation of vagal afferent signaling (Fujino et al., 2003; Tack et al., 2006; Ariga et al., 2007) and activation of the cholinergic neurons in the enteric nervous system (Edholm et al., 2004; Bassil et al., 2006). The GLRN receptor has been reported to show high levels of expression in the stomach and lower levels in the colon (Dass et al., 2003). Although there are no available data regarding the action of ipamorelin on gastrointestinal motility, we speculate that activation of GLRN receptors on enteric nerves (Dass et al., 2003; Xu et al., 2005) and on vagal afferents (Sakata et al., 2003) may accelerate gastric emptying in rats with POI. Recent studies have shown that a single intravenous infusion of ghrelin (Trudel et al., 2002) or synthetic GLRN receptor agonists, such as RC-1139 (Rejuvenon Corp., Woodlands, TX) (see Poitras et al., 2005) or TZP-101 (Venkova et al., 2007) in rats after surgery, increased gastric emptying, and small intestinal transit.

In our experiments, we found that ipamorelin caused a significant dose-dependent acceleration of colonic transit, shortening the time to the first bowel movement after surgery and significantly increasing cumulative fecal pellet output during the first 48 h. The mechanism(s) underlining the effect of ipamorelin on colonic motility are unknown, but a direct stimulation of colonic motility seems unlikely because in previous studies, ghrelin failed to induce colonic motility in vivo and had no effects on contractility studied in isolated colonic tissue (Trudel et al., 2002; Dass et al., 2003). However, it is possible that ipamorelin, like the centrally acting ghrelin receptor agonist GSK894281, may stimulate colonic transit, defeation, and increase fecal pellet output in rats by entering the central nervous system and activating GLRN receptors in the spinal cord (Shafton et al., 2009). An alternative mechanism by which ipamorelin may accelerate colonic transit could be the acceleration of gastric and small intestinal transit, which, in turn, could promote propagation of fecal matter in the colon. Such a mechanism of action is in agreement with previous research demonstrating that the peripherally restricted synthetic ghrelin receptor agonists TZP-101 could improve colonic transit and fecal output in a rat model of postoperative ileus (Frazier et al., 2009).

Another possible site of ipamorelin action is related to the potential role of ghrelin in inflammation associated with the stress response to surgery. Abdominal surgery is coupled to elevated levels of proinflammatory cytokines known to suppress GI motility (Kalff et al., 1998; de Jonge et al., 2003) and contribute to anorexia and body weight loss in experimental animals. In the present experiments, ipamorelin treatment of rats with POI improved body weight gain after surgery. Our finding is in agreement with the results of a recent study showing that postoperative ghrelin treatment decreased the production of proinflammatory cytokine interleukin-6 and stabilized the body weight in sepsis rats after surgery (Yukawa et al., 2008). However, additional studies specifically designed to investigate the effect of ipamorelin on inflammatory cytokine production are required to confirm its anti-inflammatory action.

Finally, when discussing the mechanism of action of ipamorelin in rats with POI we should consider the possibility that ghrelin receptor agonists may act as ghrelin secretagogues. Using whole-body autoradiography in the rat, Ahnfelt-Ronne et al. (2001) found that radiolabeled ipamorelin accumulates in the glandular part of the stomach, where endogenous ghrelin is synthesized and released. The possibility that ipamorelin exerts a direct action stimulating ghrelin expression in the stomach could not be excluded because administration of ipamorelin enhances the expression of ghrelin into the stomach (Yeung et al., 2006). In conclusion, our study suggests that postoperative treatment with ipamorelin administered via multiple intravenous bolus infusions over a period of 48 h may be useful in the clinic to overcome the symptoms and accelerate the recovery in patients with POI.

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