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Effect of the ghrelin receptor agonist TZP-101 on colonic transit in a rat model of postoperative ileus

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ABSTRACT

Ghrelin, the natural ligand of the growth hormone secretagogue receptor (ghrelin receptor), is an orexigenic gut hormone with prokinetic action in the upper gastrointestinal tract. Previously we have shown in a rodent model of postoperative ileus that the synthetic ghrelin receptor agonist TZP-101 prevents the delay in gastric emptying and improves small intestinal transit. The goal of the present study was to investigate whether TZP-101 affects colonic transit and food intake in rats with postoperative ileus. Fasted rats were treated with morphine and subjected to laparotomy under isoflurane anesthesia. Following surgery the animals were placed in clean home cages and fecal pellet output and food intake were monitored for 48 h. TZP-101 or vehicle were administered as 3 i.v. bolus infusions at 0 h, 2 h and 4 h post-surgery. TZP-101 (0.03–1 mg/kg) dose-dependently decreased the time to first bowel movement and increased fecal pellet output measured at 12 h and 24 h post-surgery compared to the vehicle. The administration of TZP-101 was not associated with a significant alteration in food intake. In conclusion, this study provides the first experimental evidence that a novel ghrelin receptor agonist improves large bowel function in rats with postoperative ileus, suggesting that TZP-101 may be useful in the clinic to accelerate upper gastrointestinal transit and to shorten the time to the first bowel movement following surgery.

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1. Introduction

Postoperative ileus is the temporary impairment of gastrointestinal motility following abdominal surgery. The pathogenesis of prolonged ileus is positively correlated with the degree of surgical trauma and the use of opioids for pain management (Fotiadis et al., 2004; Artinyan et al., 2005). Surgical injury triggers local immune and nerve mediated mechanisms that inhibit coordinated gastrointestinal motility (Kalff et al., 1998; Boeckxstaens et al., 1999; Boeckxstaens, 2002; Bauer et al., 2002; de Jonge et al., 2003). In addition, exogenous opioids, administered to treat post-surgical pain, prolong the duration of ileus via the activation of peripheral opioid receptors inhibiting neurally mediated contractions as well as through direct effects on central opioid receptor signaling (Gmerek et al., 1986; Greenwood-Van Meerveld, 2007). Regardless of the abdominal site of surgical intervention, the spontaneous resolution of postoperative ileus appears to be region-specific and is typically characterized by the initial recovery of the small intestine, followed by the stomach, and then the recovery of the colon (Graber et al., 1982; Waldhausen et al., 1990; Schippers et al., 1991). Since the first bowel movement is considered a clinical end point for the recovery of gastrointestinal function, assessment of the efficacy of new prokinetic drugs to reduce the time to first bowel movement following surgery in animal models is essential for the progress in postoperative therapy in humans.

Ghrelin is an acylated peptide hormone secreted predominantly from endocrine cells in the gastric oxyntic mucosa (Kojima et al., 1999; Date et al., 2000). Ghrelin receptors regulating gastrointestinal function are expressed on vagal afferents (Sakata et al., 2003; Burdyga et al., 2006) and in the enteric nervous system (Dass et al., 2003; Xu et al., 2005), where ghrelin receptor expression appears to decrease distally from high levels in the stomach to lower levels in the colon (Dass et al., 2003; Kojima and Kangawa, 2005). The prokinetic actions of ghrelin on the stomach and small intestine arise from increased signaling through vagal afferents (Fujino et al., 2003; Murray et al., 2006) and direct activation of the enteric nervous system. Furthermore, previous studies have demonstrated that the administration of the ghrelin peptide or synthetic ghrelin receptor agonists restored

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gastric motility and accelerated upper gastrointestinal transit in various rodent models of ileus (Trudel et al., 2002; De Winter et al., 2004; Poitras et al., 2005; Sallam et al., 2007). A novel macrocyclic small molecule TZP-101 with peptidomimetic effects similar to the effects of ghrelin has been developed by Tranzyme Pharma for the treatment of gastrointestinal and metabolic disorders. The structure of TZP-101 features an 18-membered macrocycle containing 3 amide bonds and a secondary amine, as well as 4 stereogenic centers (Hoveyda et al., 2006; Sandham, 2008). Pharmacodynamic analyses suggested TZP-101 expresses activity at the ghrelin receptor (Lasseter et al., 2008). Using a rat model of postoperative ileus induced by abdominal surgery and morphine treatment we found that the synthetic ghrelin receptor agonist TZP-101 normalized gastric emptying and improved small intestinal transit (Venkova et al., 2007). Since patient recovery from postoperative ileus is actually assessed by the first bowel movement following surgery, an important study is to assess whether TZP-101 reduces the time to the first bowel movement in rats with postoperative ileus. Thus the current study describes a series of experiments to investigate whether systemic TZP-101 has an effect on fecal pellet output in rats with postoperative ileus. Although, there is no evidence to support a direct effect of ghrelin in the large intestine (Trudel et al., 2002; Edholm et al., 2004; Kitazawa et al., 2005; Bassil et al., 2005), we hypothesized that a ghrelin receptor agonist may shorten the overall transit time by stimulating gastric emptying and small intestinal transit.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–350 g) were purchased from Charles River Laboratories (Wilmington, MA) equipped with a polyurethane catheter implanted into the right jugular vein. The exterior end of the catheter was secured to the dorsal neck area and used for i.v. dosing of TZP-101 or vehicle. The catheters were flushed with heparinized saline once every 3–4 days but not on the day of the experiment. Rats that were not subjected to surgery and drug or vehicle treatment were equipped with catheters implanted into the proximal colon and used to infuse a dye marker for the measurement of colonic transit time. A total of 46 rats were used in the study. All animals were housed one per cage at standard conditions (21–22 °C, 12 h light/dark cycle, controlled humidity) with water and rat chow available *ad libitum*. At least one-week acclimation to the animal facility was allowed prior to the experiments. The experimental protocol and number of animals were approved by the Animal Care and Use Committees at the V.A. Medical Center and the University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

2.2. Abdominal surgery

Prior to the experiments, rats were fasted for 20–22 h with free access to water. Postoperative ileus was induced by a surgical procedure described as "running of the bowel" (Kalff et al., 1998). Specifically, the rats were anesthetized with isoflurane (2–3% inhalation), the abdomen was shaved, disinfected and a midline incision was made to expose the abdominal viscera. The small intestine, the cecum and colon were exteriorized and inspected by gently pressing between two cotton applicators soaked in sterile saline. After completing the inspection, the intestines were covered with gauze soaked in saline and the abdomen was left open for 10 min. At the end of surgery 0.2 ml of dye (trypan-blue in saline) was carefully injected into the proximal colon (1 cm distal to the cecum) using a hypodermic needle. The viscera were then placed back into the

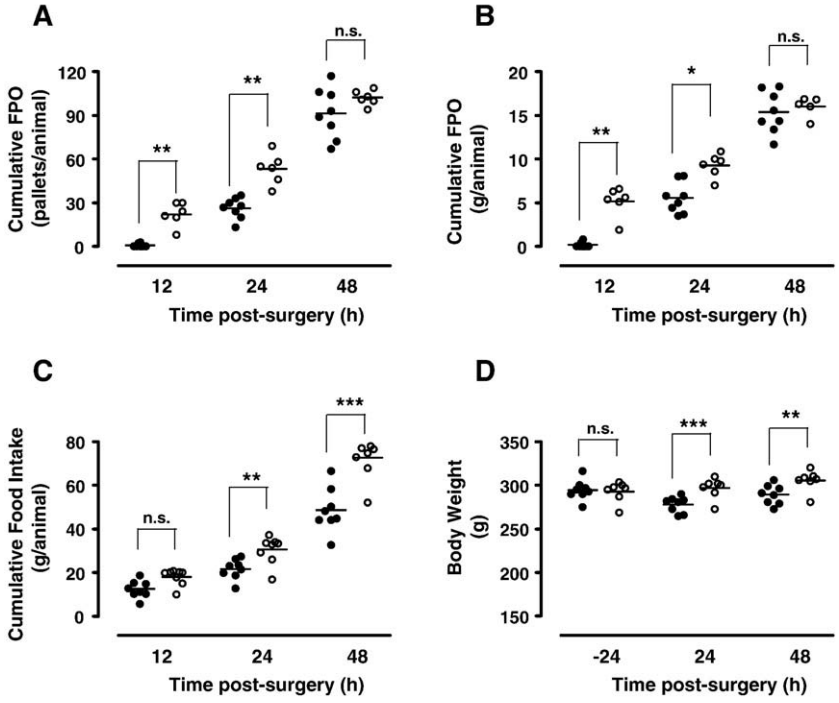

Fig. 1. Time-course of the changes in fecal pellet output, food intake and body weight in rats with postoperative ileus induced by morphine treatment followed by abdominal surgery compared to naïve rats. On the day of the experiment, all rats were fasted overnight and the naïve rats were placed in the observation cage supplied with food and water without been subjected to morphine treatment or surgery. Cumulative fecal output, expressed as the number of fecal pellets (A) or the stool weight (B), and food intake (C) were measured at 12 h. 24 and 48 h following the end of surgery. Body weight (D) was measured 24 h prior to fasting and at 24 h and 48 h of observation. Filled symbols represent individual experiments in rats with postoperative ileus while open symbols represent individual experiments in naïve rats. Mean values are from 6 naive rats and 8 rats with postoperative ileus. Statistical significance of differences was assessed by two-way ANOVA followed by Bonferonni's post test: *$P<0.05$, **$P<0.01$, ***$P<0.001$.