Ghrelin improves burn-induced delayed gastrointestinal transit in rats

H. S. Sallam,1 H. M. Oliveira,1 H. T. Gan,1 D. N. Herndon,2 and J. D. Z. Chen1
1Division of Gastroenterology, Departments of Internal Medicine and Surgery, 2Shriners Hospital for Children, University of Texas Medical Branch, Galveston, Texas

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MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (300–350 g; Harlan Sprague-Dawley, Houston, TX) were housed in wire-bottom cages in a temperature-controlled environment at 22°C, humidity 40%, and a 12:12-h light-dark cycle. Rats had free access to regular chow pellets and drinking water. There was 1 wk of acclimatization, before the initiation of the experiments. The study was approved by the Animal Care and Use Committee of the University of Texas Medical Branch (Galveston, TX). The experiments were performed in adherence to the National Institutes of Health Guidelines on the use of laboratory animals.

Experimental Procedure

Burn model. Animals, deprived of food for 24 h, were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL) inhalation (2–3%). Intramuscular buprenorphine (0.1 mg/kg, one time just before burn; Reckitt Benckiser Healthcare) was used as a pain killer in all animals. Rats were shaved on the dorsal and ventral surface of the abdomen, and a 60% total body surface area burn was inflicted by immersing the dorsal surface of the rat in 96°C heated water for 10 s and the ventral surface for 2 s according to the Walker-Mason burn model, which has been histologically studied, showing to result in a full-thickness (3rd degree) cutaneous burn (39). Burned rats were resuscitated immediately with Parkland formula (4 ml·kg−1·% total body surface area−1·24 h−1) in which a total of 24 ml of Ringer lactate solution (for the first 8 h postburn) was given intraperitoneally. The rats in the sham group were treated identically except for the burn injury, and they received 12 ml of the same solution for resuscitation. They were returned to the cage for awakening. All experiments were done 6 h after burn/sham injury.

Ghrelin. Ghrelin desiccate (1 mg; Tocris Cookson, Ellisville, MO) was dissolved in 3 ml saline and kept in ice during the experiment.

Gastrointestinal transit studies. Gastric emptying and intestinal and colonic transit studies were performed. Within each study, rats were randomized into two main groups as follows: sham injury and ghrelin-treated burn injury with doses of 0, 2, 5, and 10 nmol/rat ip 6 h after burn. Sham/burn injury was induced under anesthesia. Rats received a phenol red meal 20 min following ghrelin injection. Based on the most effective ghrelin dose, 1 mg/kg sc atropine was given 30 min before the ghrelin in one group of rats for each study. The rats in each group were killed 30–90 min later; their stomachs, intestines, and colons were harvested immediately, and the amount of phenol red recovered was measured. Percentage of gastric emptying (GE%) and geometric center for IT and CT were calculated.

We found 1) severe cutaneous burn injury significantly delayed GE, IT, and CT compared with sham injury (P < 0.05); 2) ghrelin normalized both GE and IT; but not the CT; 3) the most effective dose of ghrelin was 2 nmol/rat; and 4) atropine blocked the prokinetic effects of ghrelin on GE% and IT. In conclusion, ghrelin normalizes burn-induced delayed GE% and IT. In rats

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Ghrelin has been under intensive study for its effects on gastrointestinal motor activity and its roles in motility regulation (22, 36, 31). Not only does it influence food intake and energy balance, ghrelin also possesses prokinetic characteristics mediated through the activation of cholinergic (13, 20) and tachykinergic pathways (3).

Ghrelin has been shown to accelerate gastric emptying in postoperative (36, 37) and septic ileus (11) animal models. However, it has never been studied in a burn animal model. It is unknown whether ghrelin can exert a similar prokinetic effect on burn-induced impaired gastrointestinal motility, thus having a clinical role in treating burn patients. We aimed at testing the effect of this newly evolved stomach peptide on burn-induced delayed gastrointestinal transit in rats.

Address for reprint requests and other correspondence: J. Chen, 1108 the Strand, Rm. 221, Univ. of Texas Medical Branch, Galveston, TX 77555-0632 (e-mail: jianchen@utmb.edu).

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were divided into the following two groups: a sham burn (control) group and a burn group. Within the burn group, rats were treated with different doses of ghrelin (0, 2, 5, and 10 nmol/300- to 350-g rat) given in a random order with a total of six rats in each subgroup. A dose-response curve for ghrelin was obtained for each experiment. Based on the most effective dose of ghrelin, another group of 6 rats was added to each experiment, in which atropine (1 mg/kg sc) was given 30 min before ghrelin injection.

Gastric Emptying

After a 24-h fast, rats were exposed to sham/burn injury and injected with different doses of ghrelin (0, 2, 5, and 10 nmol/300- to 350-g rat) 6 h later. After ghrelin injection (20 min), the rats received a gavage feeding of 1.5 ml of a prewarmed methylcellulose meal mixed with Phenol red as a marker (see Composition of Phenol Red Meal).

Intestinal and Colonic Transit

After an overnight fast, rats were given general anesthesia (2–3% isoflurane inhalation) and underwent abdominal surgery. A small polyethylene tube was placed in the duodenum (or colon) via the stomach (or cecum), 1 cm distal to the pylorus (or cecocolic junction), fixed with sutures to the gut wall, and then tunneled through the abdominal wall subcutaneously and exited from the skin at the nape of the neck. Medline incision was sutured, and rats were left to recover in their separate cages. Food and water were abundantly provided. Later (3 days), the rats were exposed to sham/burn injury, followed 6 h later by intraperitoneal ghrelin administration. Later (20 min), 1.5 ml of a prewarmed (35°C) phenol red meal (see below) was injected via the implanted polyethylene tube in the duodenum (or colon).

Composition of Phenol Red Meal

Phenol red (50 mg; Sigma, St. Louis, MO) was diluted in 100 ml aqueous methylcellulose (1.5%; Fisher Scientific, Fair Lawn, NJ) solution and used as a test meal. Methylcellulose was dispersed in hot water (80°C) under continuous stirring. The solution was then allowed to cool to 35°C, and the phenol red was added. Intensity and duration (5 h) of agitation were kept constant to obtain solutions of 400 cP viscosities. This high-viscosity meal was selected to yield a transit slower than simple aqueous solutions.

Measurements of Gastric Emptying and Intestinal and Colonic Transit

Thirty minutes (or 90 min in case of colonic transit) after the test meal, the rats were killed under general anesthesia with 5% isoflurane inhalation, confirmed by chest opening. Four animals in each study without any kind of manipulations were killed immediately after the inhalation, confirmed by chest opening. Four animals in each study were divided into the following two groups: a sham burn (control) group and a burn group. Within the burn group, rats were treated with different doses of ghrelin (0, 2, 5, and 10 nmol/300- to 350-g rat) given in a random order with a total of six rats in each subgroup. A dose-response curve for ghrelin was obtained for each experiment. Based on the most effective dose of ghrelin, another group of 6 rats was added to each experiment, in which atropine (1 mg/kg sc) was given 30 min before ghrelin injection.

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Phenol Red Analysis

Phenol red analysis was done as described by Scarpignato et al. (31). Each gut segment was individually homogenized using a Fischer Scientific PowerGen 700 homogenizer with 100 ml of 0.1 N NaOH. The mixture was kept at room temperature for 1 h. Supernatant (5 ml) was added to 0.5 ml of TCA solution (20% wt/vol) to precipitate the proteins. After centrifugation (2,500 g during 20 min), the supernatant was added to 4 ml of NaOH (0.5 N) to develop the maximum intensity of color. The solutions were read using a Beckman DU 650 spectrophotometer (fixed wavelength of 560 nm).

Calculation of Percent Gastric Emptying

Gastric emptying was determined as 100% minus the percentage of gastric retention, which was defined as the ratio between the amount of phenol red recovered from the stomach 30 min after the meal and the amount of phenol red recovered from the stomach immediately after the meal.

Calculation of Small Intestinal and Colonic Transit

Small intestinal and colonic transits were assessed using a parameter called geometric center (GC), which was calculated as follows: 

\[
GC = \text{sum of } n \times P_n \text{ for } n = 1, 2, \ldots, 10
\]

Where “n” was the number of the intestinal segment and “P_n” was the percentage of phenol red recovered from the corresponding segment.

Statistical Analysis

Statistical analysis of the obtained data was executed using one-way ANOVA for multiple comparisons. Data are expressed as means ± SE. Significance was accepted at \( P < 0.05 \).

RESULTS

Effect of Burn on Gastric Emptying and Intestinal and Colonic Transits

Severe cutaneous burn injury significantly delayed gastric emptying and intestinal and colonic transits. Percentage of gastric emptying was significantly decreased in burn vs. sham burn (41.7 ± 2.1 vs. 83.7 ± 3.7, \( P < 0.05 \); Fig. 1A). The geometric center of intestinal transit was decreased significantly in burn vs. sham burn (3.6 ± 0.2 vs. 4.7 ± 0.2, \( P = 0.002 \); Fig. 1B). The geometric center of colonic transit was decreased significantly in burn vs. sham burn (2.1 ± 0.1 vs. 3.2 ± 0.3, \( P = 0.03 \); Fig. 1B).

Effect of Ghrelin on Burn-Induced Delayed Gastric Emptying

Ghrelin significantly normalized burn-induced delayed gastric emptying. The percentages of gastric emptying were 81.2 ± 2.2, 81.5 ± 5, and 71.3 ± 4.3 in 2, 5, and 10 nmol ghrelin-treated rats, respectively. The 2- and 5-nmol ghrelin doses had almost identical effects; both normalized burn-induced delay in gastric emptying. We considered the lowest dose, the 2-nmol/rat dose, as the most effective in increasing gastric emptying (Fig. 2).

Effect of Ghrelin on Burn-Induced Delayed Small Intestinal Transit

Ghrelin normalized burn-induced delayed intestinal transit. The geometric centers were 4.92 ± 1.2, 8.2, 8.0, and 4.75 ± 0.2 in 2, 5, and 10 nmol ghrelin-treated rats, respectively. As it can be seen from Fig. 3, all of these doses normalized burn-induced delayed intestinal transit. Accordingly, the 2-nmol ghrelin dose was considered as the most effective in accelerating intestinal transit.

Effect of Ghrelin on Burn-Induced Delayed Colonic Transit

Ghrelin had no effects on burn-induced delayed colonic transit. The geometric center was 2.4 ± 0.2 in 2, 5, and 71.3 nmol ghrelin-treated rats, respectively. The 2- and 5-nmol ghrelin doses had almost identical effects; both normalized burn-induced delay in intestinal transit.
and 10-nmol ghrelin-treated rats, respectively ($P > 0.05$, Fig. 4).

**Effect of Atropine on Burn-Induced Delayed Gastric Emptying and Intestinal Transit**

Atropine blocked the prokinetic effect of 2 nmol/rat ghrelin dose on gastric emptying and intestinal transit. The percentage of gastric emptying was significantly decreased from 81.2 ± 2.2 to 42.7 ± 4.4 ($P = 0.0001$; Fig. 2). The geometric center for the small intestine was significantly decreased from 4.9 ± 0.3 to 4.2 ± 0.2 ($P = 0.04$; Fig. 3).

**DISCUSSION**

In this study, we were able to demonstrate the following. 1) Severe cutaneous burn injury significantly delayed gastric emptying and intestinal and colonic transit. 2) Ghrelin at the most effective dose was able to normalize burn-induced delayed gastric emptying and intestinal transit but had no effects on colonic transit. 3) The most effective dose of ghrelin to accelerate upper gastrointestinal transit was 2 nmol/300- to 350-g rat. 4) Atropine blocked the ghrelin effect on gastric emptying and intestinal transit.

In this study, we showed that severe cutaneous burn injury significantly delayed gastric emptying and intestinal and colonic transit. These results were expected since it is known from previous reports that severe burn injury delays gastric emptying (1) and intestinal (7, 38) and colon (7, 16, 38) transits in rats. It is also known that inhibition of the gastrointestinal motility has been reported in humans following severe burn injury. Major clinical problems, including upper gastrointestinal ulcerations, bleeding, feeding intolerance, abdominal distension, vomiting, and ileus are not uncommon in this unfortunate group of patients (1, 25). Gastrointestinal complications are frequent and account for the high morbidity and mortality of burn injury.

To the best of our knowledge, this is the first time to report the effect of ghrelin on burn-induced gastrointestinal dysmotility in rats. Ghrelin is known to possess prokinetic characteristics. In healthy mice, ghrelin increased gastric emptying (2, 20, 23, 40). In healthy rats, ghrelin also improved gastric emptying (9, 15, 25, 37), increased the frequency of migrating
motor complex, and the intestinal transit (13, 14). In healthy dogs, ghrelin stimulated antral contractility and antroduodenal coordination, hence its accountability to increase gastric emptying (29). In healthy volunteer (26), normal weight (33), and gastroparetic (4, 28) human subjects, ghrelin also increased gastric emptying.

We showed that the dose required to accelerate burn-induced delayed upper gut dysmotility was 2 nmol/300- to 350-g rat. This is comparable to the ghrelin dose (20 μg/kg = 6 nmol/kg) used to obtain a prokinetic effect in other studies in rats (9, 27, 37). We would like to acknowledge that the dose of 1 nmol was not tested. However, it is unlikely that a dose of 1 nmol would be more effective than 2 nmol based on our data and available information in the literature. In our studies, we found that 5 nmol was more effective than 10 nmol. However, there was no difference in the effects between 5 and 2 nmol. Accordingly, the chance that 1 nmol is more effective than 2 nmol would be remote. Based on the reported findings in the literature, it is also unlikely that 1 nmol would be more effective. The lowest dose required to increase gastric emptying was 1.25 nmol/rat (5 μg/kg iv in 200- to 250-g normal rats; see Ref. 37). In a rodent model of postoperative ileus, gastric emptying was significantly increased only at a dose of 5 nmol/rat (20 μg/kg iv in 200- to 250-g rats; see Ref. 37). The maximum increase in the amplitude and frequency of gastric contractions was achieved only at a dose of 5 nmol/rat (20 μg/kg; see Ref. 27). When ghrelin was given intraperitoneally, only at 7 nmol/rat (20 μg/kg) was gastric emptying increased significantly. An even higher dose of ghrelin (17.5 nmol/rat or 50 μg/kg in a 250- to 350-g rat; see Ref. 10) was applied to improve intestinal transit.

We also showed that atropine blocked the 2 nmol/rat ghrelin dose effect on gastric emptying and intestinal transit, suggesting that the prokinetic effect of ghrelin was mediated via the cholinergic pathway. This result is consistent with previous reports (20, 27) in which the prokinetic effect of ghrelin was dependent on an intact vagal function (2) and that atropine and/or vagotomy blocked the effect of ghrelin on gastric (27) and intestinal motility (13) in rats. Other mechanisms involve tachykinergic pathways, as demonstrated in the electrical field stimulation studies in isolated rat stomach (3).

The exact mechanisms of burn-induced ileus are not completely understood. It has been suggested that burn-induced ileus is mediated by the nonadrenergic, noncholinergic neurotransmitter nitric oxide (NO). This has been verified by our group who reported a significant increase in both protein and mRNA of iNOS in rats after burn (16). Blocking iNOS, the mediator expressed in cells in response to stress and/or injury, accelerated delayed colon transit in rats, suggesting the involvement of NO in the pathogenesis of burn-induced ileus. Another mechanism involves COX-2 and the prostaglandin pathway. This also has been verified by our group who reported significant improvement of both delayed gastric emptying (Oliveira, unpublished observations) and colon transit (16) in rats after burn in response to treatment by selective COX-2 inhibitors.

It is unlikely that the improvement in gastric emptying by ghrelin could be explained by the protective effect of ghrelin on gastric mucosa, similar to that found in intragastric ethanol-treated and ischemia/reperfusion rat models (5, 36). Based on our observations, no macroscopic gastric mucosal injury was seen in our acute burn model (6 h after burn; Sallam, unpublished observations). Also, based on our knowledge, ghrelin triggers the release of growth hormone, and, in our experience, growth hormone can increase intestinal homeostasis after burn injury independent of epithelial cell proliferation (18). Furthermore, it remains controversial whether ghrelin can exert a protective effect on gastric mucosa, although other reports suggest that ghrelin might induce gastric mucosal lesion in rats by increasing acid secretion (19). Ghrelin effect on acid secretion remains a controversial issue itself (8, 12, 27, 32).

Ghrelin had no effect on colon transit. This is similar to the results seen in a rodent model of postoperative ileus (37). We believe that this might be related to the distribution of ghrelin receptors along the gut, which decreases distally (8, 22). It should be mentioned that only a higher dose of ghrelin (10 nmol/rat) was tested. However, based on dose-dependency studies with gastric emptying and intestinal transit, a lower dose of ghrelin would have similar results.

Based on this study, ghrelin yields a strong potential for treatment of patients with burn-induced delayed gastrointestinal transit. Clinically, improvement of gastrointestinal transit will facilitate enteral resuscitation, correcting patients’ hypovolemia and reducing patients’ symptoms. Economically, this would reduce the duration of hospital stay and the costs of health care for burn patients (25).

In conclusion, ghrelin normalizes burn-induced delayed gastric emptying and intestinal transit in rats, an action mediated via the cholinergic pathway. Ghrelin may have a therapeutic potential for burn patients with delayed upper gastrointestinal transit. Further studies are needed to investigate other mechanisms of action through which ghrelin exerts its prokinetic action in burn.

REFERENCES


