Ghrelin improves burn-induced delayed gastrointestinal transit in rats

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gastrointestinal transit is common in patients with severe burn. Ghrelin is a potent prokinetic peptide. We aimed at testing the effect of ghrelin on burn-induced delayed gastrointestinal transit in rats. Gastric emptying (GE), intestinal transit (IT), and colonic transit (CT) studies were performed in male Sprague-Dawley rats. 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 but has no effect on CT in rats. The prokinetic effects of ghrelin are exerted via the cholinergic pathway. Ghrelin may have a therapeutic potential for burn patients with delayed upper gastrointestinal transit.

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 tachykininergic 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.

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; Toscris Cookson, Ellsiville, 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

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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 meal to serve as controls. For gastric emptying, the entire stomach was carefully isolated, ligated just above the cardia and below the pylorus, and removed. For the intestinal transit, the entire small intestine was carefully harvested and divided into 10 equal segments; the stomach and colon were harvested at the same time as a control for simple aqueous solutions. 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 immediately after the meal and the amount of phenol red recovered from the stomach immediately after the meal.

**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 = sum of n × Prn for n = 1, 2, . . . , 10. Where “n” was the number of the intestinal segment and “Prn” 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 ± 0.3, 4.73 ± 0.3, 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 and 2.1 ± 0.1 in 0-
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, suggest-
ing 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 com-
pletely understood. It has been suggested that burn-induced ileus is mediated by the nonadrenergic, noncholinergic neuro-
transmitter 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 in-
volvement 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 re-
ported 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, unpub-
lished 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). Further-
more, 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 secre-
ction 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’ hypo-
volemia 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 gas-
tric 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 mecha-

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