Clinical effects of ghrelin on gastrointestinal involvement in patients with systemic sclerosis

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Abstract. The majority of patients with systemic sclerosis (SSc) have gastrointestinal (GI) tract involvement, but therapies using prokinetic agents are usually unsatisfactory. Ghrelin stimulates gastric motility in healthy human volunteers. In this study, we investigated whether ghrelin could improve gastric emptying in patients with gastrointestinal symptoms due to SSc. The study was performed in a randomized, double-blind, placebo-controlled crossover fashion on two occasions. Ten SSc patients with GI tract involvement received an infusion of either ghrelin (5.0 µg/kg) or saline, and gastric emptying rate was evaluated by 13C-acetic acid breath test. Gastric emptying was significantly accelerated by ghrelin infusion in patients with SSc (ghrelin vs. saline: 43.3 ± 11.4 min vs. 53.4 ± 5.4 min, P=0.03). No serious adverse effects were observed. Our results suggest that ghrelin might represent a new therapeutic approach for GI tract involvement in patients with SSc.

Key words: Gastric motility, Ghrelin, Systemic sclerosis

SYSTEMIC SCLEROSIS (SSc) is a progressive and multisystem disease characterized by microvascular damage and excess deposition of connective tissue in skin and internal organs, including kidneys, heart, lungs, and gastrointestinal tract. Therefore, patients with SSc have various symptoms such as Raynaud’s phenomenon, thickened or hardened skin, and scleroderma renal crisis. In addition to these symptoms, any part of the gastrointestinal (GI) tract can be affected in patients with SSc, and they often have dysphagia, heartburn, bloating, abdominal pain, and diarrhea [1, 2]. Although involvement of the GI tract is not a direct cause of death, it leads to a decline in the quality of life. In an autopsy study, GI muscle atrophy and fibrosis (both of which lead to decreased GI motility) were detected in the esophagus, small intestine, and colon in 74%, 48%, and 39% of patients, respectively [3]. In previous studies, 50–67% of patients with SSc report delayed gastric emptying, which correlates with symptoms of early satiety, bloating, and emesis [4-12]. At present, the cause of scleroderma is still unknown, and there are no effective treatments for SSc. Consequently, the various complications of SSc are treated individually. To treat gastroparesis, prokinetic agents such as metoclopramide, domperidone, erythromycin, mosapride citrate, dinoprost, and octreotide have been used in an attempt to improve GI motility; however, therapies with these agents are usually unsatisfactory [13-17].

Ghrelin, a gut hormone that is produced mainly in the stomach, is a 28–amino acid peptide with an n-oc-
tanylation modification at Ser\textsuperscript{3} \cite{18, 19}. This peptide stimulates gastric motility and accelerates postprandial gastric emptying in human volunteers \cite{20}. Acceleration of gastric emptying by ghrelin has also been observed in patients with diabetic, idiopathic, and post vagotomy gastroparesis, although the numbers of patients enrolled in those studies were small \cite{21-23}. In addition, ghrelin stimulates food intake following peripheral administration \cite{24, 25}. Therefore, we hypothesized that ghrelin might be useful for the treatment of GI disorders related to SSc.

In this study, we investigated whether administration of ghrelin could improve gastric emptying in patients with GI symptoms due to SSc. We also evaluated the safety of ghrelin injection to treat GI involvement in patients with SSc.

**Subjects and Methods**

**Patients**

To be included in this study, subjects had to have SSc as defined by the American College of Rheumatology (ACR) (Table 1) and exhibit GI involvement. Criteria for GI involvement included gastroesophageal reflux disease (GERD), dysphasia, early satiety, postprandial fullness, bloating, bacterial overgrowth requiring antibiotics, abdominal pain, diarrhea, and/or malabsorption syndrome (Table 2). Exclusion criteria were 1) localized scleroderma; 2) esophageal stenosis; 3) receiving parenteral or enteral nutrition; 4) past history of open-abdominal surgery of the GI tract; 5) allergy against milk or liquid meal (Racol\textsuperscript{TM}); 6) severe hepato-renal or respiratory disorders, severe depression, schizophrenia, mania, severe diabetes, congenital aminoacid metabolic disorder; 7) tendency or past history of suicide; 8) pregnancy; 9) lactation; and 10) past history of malignant tumors. Medication that had already been started before the initial enrolment could be continued during this study. During the entire period of this study, any additional drugs that might influence the study outcome, including prokinetics, antipeptic ulcer agents, and drugs to treat intestine, liver, gallbladder, or pancreatic disease, were not allowed at any time. The initial planned sample size was ten or more. Patient registration lasted from Oct 2010 to Aug 2011. The study protocol was approved by the Ethics Committees on Human Research of the Kyoto University Graduate School of Medicine. We obtained written informed consent from all subjects prior to enrolment. This study was conducted according to the Declaration of Helsinki principals. This trial was registered at the UMIN Clinical Trials Registry as UMIN000003739.

**Study design**

The study was performed in a randomized, double-blind, placebo-controlled two-period crossover fashion on two occasions with a washout interval of at least 2 weeks (Fig. 1). Medication that might affect gastric motility (e.g.: metoclopramide, anticholinergics, calcium channel antagonists, macrolide antibiotics) was discontinued at least 24 hours before \textsuperscript{13}C-acetic acid breath test. After a 12-hour fast, SSc patients underwent \textsuperscript{13}C-acetic acid breath test; breath testing started between 8:30 and 9:00 am. Liquid meal (Racol\textsuperscript{TM}, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was used as the test meal. The nutrient composition of 100 mL of liquid meal (100 kcal) is 4.4 g of protein, 15.6 g of carbohydrate, and 2.2 g of fat. \textsuperscript{13}C was used to label

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Criteria for the Classification of Systemic Sclerosis (Defined by the American College of Rheumatology [ACR])</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major criterion:</strong></td>
<td>Proximal diffuse (truncal) sclerosis (skin tightness, thickening, non-pitting induration)</td>
</tr>
<tr>
<td><strong>Minor criteria:</strong></td>
<td>Sclerodactyly (only fingers and/or toes)</td>
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<td></td>
<td>Digital pitting scars or loss of substance of the digital finger pads (pulp loss)</td>
</tr>
<tr>
<td></td>
<td>Bilateral basilar pulmonary fibrosis</td>
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<tr>
<td>The patient should fulfill the major criterion or two of the three minor criteria.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Grading of Gastrointestinal Involvement in Systemic Sclerosis (Draft Guidelines of Japan)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper Digestive Tract</strong></td>
<td></td>
</tr>
<tr>
<td>1. Normal</td>
<td></td>
</tr>
<tr>
<td>2. Mild: No symptom, but with decreased peristalsis in the lower esophagus</td>
<td></td>
</tr>
<tr>
<td>3. Moderate: Gastroesophageal Reflux Disease (GERD)</td>
<td></td>
</tr>
<tr>
<td>4. Severe: Dysphagia caused by reflux esophagitis</td>
<td></td>
</tr>
<tr>
<td>5. Very Severe: Dysphagia caused by esophageal stenosis</td>
<td></td>
</tr>
<tr>
<td><strong>Lower Digestive Tract</strong></td>
<td></td>
</tr>
<tr>
<td>1. Normal</td>
<td></td>
</tr>
<tr>
<td>2. Mild: Intestinal lesions such as bloating, abdominal pain, diarrhea, etc. with no need for antibiotics</td>
<td></td>
</tr>
<tr>
<td>3. Moderate: Overgrowth of enteric bacteria with need for antibiotics</td>
<td></td>
</tr>
<tr>
<td>4. Severe: Chronic intestinal pseudo-obstruction or malabsorption syndrome</td>
<td></td>
</tr>
<tr>
<td>5. Very Severe: Received intravenous hyperalimentation</td>
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</table>
Acetate (99%; Cambridge Isotope Laboratories, Woburn, MA, USA), which is absorbed in the duodenum but not in the stomach. Liquid meal was mixed with 13C-acetate (100 mg), and all patients ingested 200 mL of test meal within a few minutes. Ghrelin (5.0 µg/kg) or placebo was injected intravenously over 30 seconds immediately after ingestion of the test meal. Breath samples were collected for 13CO2 measurement before the meal, and then every 15 minutes for 3.5 hours. During each expiration phase, exhaled air was collected into a bag a total of 15 times. The concentration of 13CO2 was measured using a gas chromatograph isotope-ratio mass spectrometer (UBit-IR300, Ootsuka Electronic Corp), and the measured values were presented as the delta-13CO2 (expressed as a percentage). To evaluate the effect of ghrelin on gastric emptying, T_max was defined as the time taken to reach the maximum concentration, calculated using the delta-13CO2 values.

**Tmax assessed by 13C-acetate breath test**

The 13C-acetate breath test was developed as a non-radioactive alternative for the measurement of gastric emptying. Braden and colleagues [26] reported that half-emptying times for the 13C-acetate breath test were closely correlated with those measured by radioscintigraphy, using both semisolids and liquids, and that the T_max of 13CO2 exhalation was itself a reliable parameter compared with the half-emptying times obtained by scintigraphy.

**Primary endpoint**

The primary endpoint of this trial was Tmax assessed by 13C-acetic acid breath test.

**Secondary endpoint**

The secondary endpoints of this trial were alteration in hunger sensation and serum GH levels upon ghrelin or placebo administration.

**Assessment of safety**

Vital signs, including blood pressure, pulse rate, and body temperature, were measured during examination. Changes in physical symptoms were assessed by phone interview 2 weeks after ghrelin or saline injection. If the study was interrupted, assessments of safety by...
hematology, blood chemistry, and urine analysis were performed when the study was discontinued.

**Ghrelin**

Human ghrelin was prepared as described previously [28]. Ghrelin was dissolved in 3.75% D-mannitol to a final concentration of 180 μg/mL. Solutions were filtered and stored at −20°C in sterile vials. Examination by the Japan Food Research Laboratories (Tokyo, Japan) did not find any traces of endotoxin in the ghrelin solutions; a pyrogen test based on the Pharmacopoeia of Japan was also negative.

**Statistical evaluations**

A sample size of 10 patients was required to provide at least 90% power to detect a T_{max} ratio of 0.70 between treatments with a standard deviation of 30 minutes and an intra-patient correlation coefficient of 0.50 or more. This ratio was based on data from previous studies. All statistical analysis of the two-period cross-over design was performed using a linear mixed-effect model with treatment period as fixed effects and patient as the random effect. The 95% confidence intervals (CIs) for group means and treatment differences were estimated using least-squares means (LS-means) and robust variance. Carry-over effects were assessed using the test for the treatment by period interaction term in linear mixed-effect models. All statistical analyses were performed using the SAS software, version 8 (SAS Institute Inc., Cary, NC, USA). A two-tailed P-value was used, with the required level of significance set at 0.05.

**Results**

**Baseline characteristics of subjects**

Ten subjects (seven women, median age 67.5 years, range 48–80 years) were enrolled in this study (Table 3). All ten subjects were diagnosed with SSc, as defined by the criteria of the American College of Rheumatology (ACR); all subjects fulfilled the major criterion. Three subjects had diffuse SSc, and seven had limited SSc. Anti–topoisomerase 1 antibodies were positive in two patients, and anti-centromere antibodies were positive in four patients. As shown in Table 3, all subjects exhibited GI involvement; nine had early satiety and postprandial fullness, seven had heart burn and dysphagia caused by reflux esophagitis, and two had diarrhea. Average BMI at registration was 21.1 ± 4.2 kg/m². None took H2-blockers or antibiotics. One patient (Patient No. 2) discontinued this examination after the first injection (saline) because of a gall-bladder stone attack.

**Clinical effects**

Plasma levels of ghrelin 30 minutes were 999.2 ± 23.7 fmol/mL after ghrelin injection (pre-injection, 20.2 ± 10.3 fmol/mL) and 15.4 ± 6.0 fmol/mL after saline injection (pre-injection, 18.8 ± 7.4 fmol/mL). As shown in Fig. 2, serum GH levels were significantly elevated after ghrelin administration (ghrelin vs. saline: 61.9 ± 9.5 vs. 1.4 ± 1.2 ng/mL).

Ghrelin shortened gastric emptying time in patients with SSc (Fig. 3). Gastric emptying, as determined by 13C-acetic acid breath test, was signifi-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age</th>
<th>BMI</th>
<th>subtypes</th>
<th>AT1A</th>
<th>ACA</th>
<th>Gastrointestinal Symptoms</th>
<th>Gastrointestinal Drugs</th>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>Postprandial Fullness</td>
<td>Dysphagia</td>
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<td>1</td>
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<tr>
<td>2</td>
<td>M</td>
<td>48</td>
<td>21.2</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>4</td>
<td>F</td>
<td>57</td>
<td>22.2</td>
<td>limited</td>
<td>-</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>71</td>
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<td>6</td>
<td>M</td>
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<td>+</td>
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<tr>
<td>7</td>
<td>F</td>
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<tr>
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<td>F</td>
<td>80</td>
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<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>50</td>
<td>18.8</td>
<td>limited</td>
<td>-</td>
<td>+</td>
<td>-</td>
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ACA, anti-centromere antibody; AT1A, anti–topoisomerase 1 antibody
Effects of ghrelin on gastrointestinal involvement

*Effects of ghrelin on gastrointestinal involvement*

Significantly accelerated by ghrelin injection (43.3 min, 95% CI 31.8–54.7 min) relative to saline injection (53.4 min, 95% CI 47.4–59.3 min; treatment difference −10.1 min, 95% CI −19.1 to −1.2 min, *P*=0.03). There was no carry-over effect in the primary endpoint (*P*=0.17 by interaction test). The effects of ghrelin on appetite were assessed by changes in the visual analog scores (VAS) before and after ghrelin or placebo injection. Delta-VAS was calculated as the maximum value after administration minus the value before administration. Thus, the larger amounts of changes in VAS indicated an improvement in early satiety after ghrelin or saline injection. As shown in Fig. 4, however, administration with ghrelin did not improve early satiety. No significant carryover effect or interaction effect was observed (*P*=0.06).

**Adverse effects**

No serious adverse effects were observed. We observed two moderate events, flushing and sweating, with incidences similar to those previously reported. These complaints were transient and well tolerated.

**Discussion**

Administration of ghrelin induces the migrating motor complex in fasting rat and human and accelerates postprandial gastric motility in healthy humans and patients with idiopathic, neurogenic, or diabetic gastroparesis [21-24]. In previous studies, T_{1/2 liq} (half-emptying time for liquids, in minutes) decreased by 20–30% following injection of 0.7–5.9 µg/kg of ghrelin. We previously reported that ghrelin tended to increase appetite in a dose-dependent manner (i.e., more so at 5.0 than 1.0 µg/kg) in a phase I study, and that it is safe at a dose of 5.0 µg/kg [27, 28, 31]. Based on these findings, we adopted the present protocol.

This study is the first clinical investigation to demonstrate that a single administration of ghrelin significantly accelerated gastric emptying time, relative to placebo, in patients with SSC who had current symptoms suggestive of gastroparesis; the improvement rate was about 23%. This observation is in line with animal studies and with previous reports of a stimulatory effect of a similar dose of ghrelin on gastric motility in humans [20-25]. We initially expected that administration of ghrelin would improve GI motility, result in relief of GI symptoms. However, although the gastric emptying rate was increased following ghrelin
administration, post-prandial satiety did not improve. Franck-Larsson et al. reported that delayed gastric emptying in SSc did not relate to gastrointestinal symptoms or myoelectric gastric activity [5]. Taken together, these observations suggest that factors other than gastric motility might contribute to upper gastrointestinal symptoms.

Several prokinetic agents, such as metoclopramide, domperidone, erythromycin, mosapride citrate, and dinoprost, have been used in attempts to improve GI motility in patients with SSc who suffered from gastroparesis; however, therapies with these agents are usually unsatisfactory [13-17]. Soudah et al. had reported that octreotide stimulates intestinal motility and reduces bacterial overgrowth, resulting in improvement in abdominal symptoms [15]. Based on that report, we predict that octreotide could be adapted to the treatment of gastric involvement in patients with SSc. Like octreotide, ghrelin may also have therapeutic potential for the treatment of GI dismotility in patients with SSc. Because this study was designed with a single-dose and single-injection protocol, larger-scale and longer-term prospective cohort studies are needed to define the effects of ghrelin on gastric motility in patients with SSc. Also, future studies should investigate whether ghrelin treatment can improve gastrointestinal symptoms.

Cohen et al. proposed a two-stage process in the pathophysiology of SSc: a neuropathic phase followed by a myopathic phase [32]. The second phase is characterized by smooth-muscle atrophy and replacement of muscle tissue with fibrosis. From that perspective, it is likely that ghrelin would not be effective for the treatment of SSc patients with second-phase gastroparesis. Although experiments in chemically or surgically vagotomized animals have suggested that the motility effects of ghrelin are caused mainly by activation of vagal afferents [33, 34], the ghrelin receptor is expressed in the enteric nerve system [35, 36]; furthermore, in vitro, ghrelin increases electrically induced contraction of rat and mouse muscle strips [37-40]. In addition, in previous studies, injection of ghrelin accelerated gastric motility in patients with neurogenic and diabetic gastroparesis [21-24]. Thus, we expect that ghrelin, at least, is effective for the treatment of SSc patients with first-phase gastroparesis.

There are some limitations in the present study such as small sample size, single dose and single attempt for ghrelin infusion. However, the results of this study suggest that ghrelin may have therapeutic potential for the treatment of GI dismotility in patients with SSc. Further studies using more subjects and with multiple injections of ghrelin will be required in order to confirm the effects of ghrelin on gastric motility and gastrointestinal symptoms in patients with SSc.

Acknowledgements

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Conflict of Interest

The authors have no conflict of interest and nothing to disclose.

References


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