

The prokinetic effect of mosapride citrate combined with omeprazole therapy improves clinical symptoms and gastric emptying in PPI-resistant NERD patients with delayed gastric emptying

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Abstract

Background Previous studies have shown that non-erosive reflux disease (NERD) patients are less sensitive to proton pump inhibitor (PPI) treatment than patients with erosive reflux disease. The aim of this study was to investigate whether treatment with prokinetics in addition to omeprazole therapy would improve clinical symptoms, gastric emptying and esophageal peristalsis in PPI-resistant NERD patients with or without delayed gastric emptying. **Methods** Subjects were 64 consecutive patients presenting with typical symptoms of PPI-resistant NERD ($n = 44$) and 20 healthy volunteers. PPI-resistant NERD patients underwent mosapride citrate (15 mg/day) and omeprazole (20 mg/day) co-therapy for 12 weeks. We evaluated the clinical symptoms as well as gastric emptying and esophageal manometry before and after combined therapy. We measured both acylated- and des-acylated plasma ghrelin levels by the ELISA method. The primary endpoint was to investigate whether co-administration of mosapride citrate and omeprazole would improve clinical symptoms and gastric emptying in PPI-resistant NERD patients with delayed gastric emptying.

Results T_{\max} value in PPI-resistant NERD patients was significantly higher than in healthy volunteers. Combination therapy with the prokinetic agent mosapride citrate and omeprazole significantly improved reflux symptoms and T_{\max} value in $T_{\max} > 65$ min NERD patients. Co-therapy

also significantly reduced des-acylated-ghrelin levels in NERD patients with delayed gastric emptying.

Conclusions Administration of mosapride citrate in addition to omeprazole improved gastro-esophageal reflux and gastric emptying in PPI-resistant NERD patients with delayed gastric emptying.

Keywords Non-erosive reflux disease · 5-HT₄ receptor agonist · Acylated-ghrelin · Gastric emptying

Introduction

About one-fifth of the adult population in Western countries experiences typical weekly symptoms of gastro-esophageal reflux disease (GERD), such as heartburn and regurgitation [1]. Recently, we have come to realize that the majority of GERD patients do not have any endoscopically visible lesions in the distal part of the esophagus. Several mechanisms have been proposed for the pathogenesis of non-erosive reflux disease (NERD), including visceral hypersensitivity, prolonged contraction of the esophagus and psychological factors [2–4]. Therefore, it is clear that NERD is an umbrella concept covering heterogenous subgroups of patients with different mechanisms responsible for their symptoms. It has been shown that between 33 and 50% of NERD patients presenting with heartburn do not have any evidence of pathological acid reflux during 24-h esophageal pH testing [5, 6]. Indeed, several studies have demonstrated that NERD patients are less sensitive to proton pump inhibitor (PPI) treatment than patients with erosive reflux disease [7–9].

In fact, some recent studies have suggested that the pathogenesis of NERD may differ from that of erosive

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GERD or Barrett's esophagus [10, 11]. The physiology of the lower esophageal sphincter, so fundamental to the pathophysiology of all manifestations of GERD, is intimately related to that of the gastric fundus and cardia. In other studies, gastric distention significantly increased the transient lower esophageal sphincter relaxation (tLESR) [11–13]. To date, there are no available data regarding the relationship between gastric motility and NERD patients.

Mosapride citrate is a prokinetic agent known to stimulate the serotonin 5-hydroxytryptan-4 (5-HT₄) receptor, increase acetylcholine release from the parasympathetic nerve endings, and promote bowel motility and gastric emptying [14]. Some previous studies have revealed that mosapride citrate decreases acid reflux to the esophagus in GERD patients and improves gastric emptying in healthy volunteers and diabetic patients for both solids and liquids [15, 16]. Therefore, in this study, we investigated whether mosapride citrate and omeprazole co-therapy could alleviate clinical symptoms in PPI-resistant NERD patients by improving gastric emptying and esophageal peristalsis.

Materials and methods

Patients

Forty-four consecutive patients presenting with typical symptoms of PPI-resistant NERD and 20 healthy volunteers were enrolled after upper gastrointestinal endoscopy and abdominal ultrasonography. Participants were recruited from September 2006 to April 2008. The diagnosis of NERD was based on typical reflux-associated symptoms (QUEST score >4 points) that occurred at least twice a week for at least 6 months before diagnosis in the absence of visible esophageal mucosal breaks at endoscopy. The exclusion criteria included severe heart disease, renal or pulmonary failure, liver cirrhosis, severe systemic illness and history of malignant disease. Patients with previous gastroduodenal surgery and those on recently used NSAID or anticoagulant medication were also excluded. *H. pylori* infection was determined by both the ¹³C-urea breath test and histological identification. Healthy volunteers were recruited from a general pool of medical students and doctors. Written informed consent was obtained from all subjects prior to undergoing upper gastrointestinal endoscopy and abdominal ultrasonography for evaluation of dyspeptic symptoms. The study protocol was approved by the Ethical Review Committee of the Nippon Medical School Hospital.

Study protocol

Proton pump inhibitor-resistant NERD was diagnosed when 4-week omeprazole treatment (20 mg/day) failed to

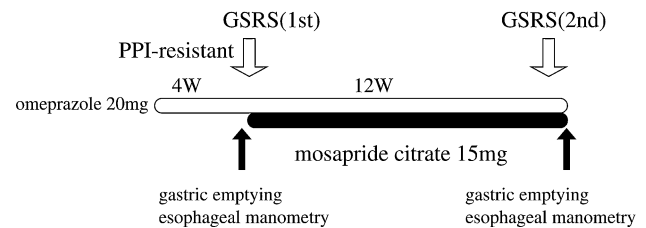


Fig. 1 Study protocol. PPI-resistant NERD was diagnosed when 4-week omeprazole treatment (20 mg/day) failed to improve clinical symptoms in NERD patients. PPI-resistant NERD patients were treated for 12 weeks with combined mosapride citrate (15 mg/day) and omeprazole (20 mg/day) therapy. Clinical symptoms, gastric emptying and esophageal manometry were evaluated before and after combined therapy

improve clinical symptoms (QUEST score >4 points) in NERD patients. PPI-resistant NERD patients were treated with the prokinetic agent mosapride citrate for 12 weeks in addition to omeprazole (20 mg/day). We evaluated clinical symptoms as well as gastric emptying and esophageal manometry twice in this study; the first evaluation was done after 4-week omeprazole treatment prior to mosapride citrate co-therapy, and the second evaluation after 12-week mosapride citrate (15 mg/day) administration in addition to omeprazole (20 mg/day) (Fig. 1). All study personnel and participants were blinded to treatment assignment for the duration of the study. In addition, we evaluated gastric emptying and esophageal manometry blindly.

Endoscopic assessment of NERD

The patients were evaluated by endoscopy for reflux-associated disease if they complained of typical heartburn symptoms, i.e., a burning feeling rising from the stomach or lower chest up toward the neck at least twice a week [17] for a period of at least 6 months prior to examination.

Clinical symptoms

GI symptoms were assessed based on the GSRs questionnaire, which proved reliable and valid for the evaluation of GI symptoms [18, 19]. The GSRs consists of 15 items, each rated on the 7-point Likert scale (1–7), ranging from “no discomfort at all” to “very severe discomfort.” The scores for abdominal pain, reflux, diarrhea, indigestion and constipation subscales were calculated by averaging the scores of the items completed within an individual subscale, with higher scores indicating more severe symptoms. After combined therapy, patients experiencing no discomfort at all for a particular symptom were considered cured for that clinical symptom; that is, when the GI score for each symptom such as abdominal pain, reflux, diarrhea, indigestion, and constipation improved to a score 1.

Measurement of gastric motility

Sodium acetate (water soluble) for emptying of liquids was used as a tracer (Cambridge Isotope Laboratories; Cambridge, MA). Probes were analyzed by non-dispersive infrared spectroscopy (IRIS, Wagner Analyzentechnik; Bremen, Germany). The subject's own production of 300 mmol CO₂ per m² body surface and per hour was set as default value. Integrated software solutions calculated the half gastric emptying time ($T_{1/2}$) and the lag phase (T_{\max}) as the point of maximum gastric emptying according to Hellmig et al. [20]. Hellmig et al. [20] have also reported that the gastric emptying of liquid meals was similar to that of solid meals. T_{\max} value >65 min indicated disturbance of gastric emptying [21].

Study protocol for gastric emptying of liquids

The liquid test meal consisted of 100 mg of ¹³C-acetate dissolved in 200 ml of a liquid meal (Racol, 1 ml/1 kcal; Otsuka Pharmacia Company; Tokyo, Japan). Breath samples were taken at 0, 10 s, 5, 10, 15, 20, 30, 40, 50, 60, 75 and 90 min after ingestion. The patients were instructed not to drink, eat or smoke during the test.

Recording methods

Esophageal manometry was performed with a 21-channel manometric assembly (Dentsleeve; Wayville, Australia). We used ten-side holes, which were spaced at 1-cm intervals starting at 3 cm above the distal end of the assembly to monitor the pressure from the proximal stomach, LES and distal esophagus. We used another seven side holes, spaced at 2-cm intervals, to monitor the pressure from the distal to the proximal esophagus and four side holes at 3, 6, 10, and 13 cm above the most proximal of the 2-cm interval side holes to monitor the pressure from the proximal esophagus to the pharynx. Each lumen was perfused with degassed distilled water at 0.15 ml/min by a low compliance manometric infusion pump (Dentsleeve). The data were digitized with a computer, and the digitized signals were displayed, stored and analyzed using Trace Software (Dr GS Hebbard, Royal Melbourne Hospital; Melbourne, Australia).

Study protocol for esophageal manometry

The subjects were examined in the sitting position after fasting overnight. The manometric assembly was passed via an anesthetized nostril and positioned so that the most proximal of the 1-cm interval side holes was 2 cm above the LES. The subjects were then allowed to adapt to the assembly for 10 min. Baseline recordings of LES pressure were made for 5 min, and then primary and secondary

peristalsis was assessed. The primary peristalsis was assessed in response to ten swallows of a 5-ml water bolus. Each swallow was separated by a 30-s interval. Secondary peristalsis was triggered by esophageal distention using a 20-ml air bolus, which was injected rapidly by hand into the mid-esophagus through an infusion port located 11 cm above the LES. After 20 s, each stimulus was followed by a dry swallow to clear any residual air. Each stimulus was repeated five times [22].

Intraluminal esophageal pH recording

Twenty-four-hour esophageal pH values were monitored in nine PPI-resistant NERD patients with delayed gastric emptying who consented to the monitoring for assessment of the severity of acid reflux in the esophagus. The study was carried out on an outpatient basis, after at least 6 h of fasting. A Digitrapper Mark III recorder (Synectics Medical, Stockholm, Sweden) was used. An antimony pH catheter (Zenetics Medical; Salt Lake City, UT) was inserted intranasally and positioned 5 cm above the lower esophageal sphincter. Patients then underwent 24-h pH monitoring, and diaries were provided for them to record their symptoms during the study period. At the end of the 24-h recording period, data were transferred to a commercially available software program (Esophagogram, Gastrosoft; Stockholm, Sweden). Patients were encouraged to maintain normal activity and sleep schedules. Meals (total 1,500 kcal, fat 25%) were given at 9:00 a.m. (breakfast), 12:30 p.m. (lunch) and 7:30 p.m. (supper).

Data analysis

The primary and secondary peristalsis was classified as successful if a pressure wave of more than 12 mmHg at the three proximal esophageal recording sites (16, 19 and 22 cm above the LES) and of more than 25 mmHg at the middle and distal esophageal recording sites progressively traversed all of the esophageal recording sites. Peristaltic progression was defined as a peristaltic velocity of <6 cm/s. The criteria of failed peristalsis were failure of a pressure wave of more than 12 mmHg at the three proximal esophageal recording sites, failure to traverse each of the esophageal recording sites, or a peristaltic velocity of more than 6 cm/s between the recording sites at 2 and 22 cm above the LES [15]. Normal secondary peristalsis was defined as the occurrence of four or more complete peristaltic responses to the five air boluses [17].

Tracings were reviewed, meals periods were timed, changes in body position were noted and the time that symptoms were recorded was compared with the information written in the diaries. The pathological acid reflux (abnormal acid exposure time) is considered present when intra-esophageal pH falls below 4 for >4.2% of the time

[23]. Analysis of pH tracing was done as follows: (1) the time period percentage with an esophageal pH of <4.0 was calculated as a proportion of the recording periods and (2) acid reflux was defined as those periods when the pH in the esophagus dropped to below 4.0.

Measurement of plasma ghrelin levels

We then measured the plasma ghrelin levels to evaluate their association with gastric motility. Blood was drawn into chilled tubes containing EDTA-2Na (1 mg/ml) and aprotinin (500 U/ml), and the plasma was taken after immediate centrifugation and stored at -30°C until assay. Plasma immunoreactive ghrelin concentrations (acylated- and des-acylated) were measured in duplicate using a commercial radioimmunoassay (Phoenix Pharmaceuticals; Belmont, CA). The intra-assay coefficient of variation (CV) was 8.1%, and the inter-assay CV, 9%. The range of detection was 10–1,280 pg/ml.

Measurement of serum pepsinogen I and II

Serum PGI and II levels were measured using E Plate Pepsinogen I and II (Eiken Chemical Co., Ltd.) using cutoff levels of $\text{PGI} < 70 \mu\text{g/L} + \text{PGI/II ratio} < 3$ as low PG levels (L-PG) and $\text{PGI} < 30 \mu\text{g/l} + \text{PGI/II ratio} < 2$ as very low PG levels (VL-PG) according to the instructions of the manufacturer.

Statistical analysis

For statistical evaluation of group data, Student's *t* test for paired data and analysis of variance (ANOVA) for multiple comparisons were followed by Scheffe's *F* test. Mann-Whitney *U* test was used for analysis of categorical data. A *P* value of <0.05 was considered statistically significant.

Results

Characteristics of PPI-resistant NERD patients

BMI scores did not vary significantly between the two groups of patients and healthy volunteers (Table 1). There

Table 1 Characteristics of the patients

	Sex	Age	BMI	HP positivity (%)
Healthy volunteer (<i>n</i> = 20)	M 14 F 6	46.2 ± 12.8	21.8 ± 1.2	15
PPI-resistant NERD (<i>n</i> = 24) ($T_{\text{max}} > 65$ min)	M 10 F 14	44.2 ± 8.1	22.9 ± 2.1	33
PPI-resistant NERD (<i>n</i> = 20) ($T_{\text{max}} < 65$ min)	M 12 F 8	41.2 ± 7.9	23.1 ± 1.7	30

was also no significant variation in *H. pylori* positivity rates between PPI-resistant NERD patients with delayed gastric emptying and those without (Table 1). The pepsinogen I/II ratio in PPI-resistant NERD patients with or without delayed gastric emptying and healthy volunteers was 5.68 ± 2.4 , 5.72 ± 1.9 and 6.71 ± 1.8 , respectively. There was no significant difference in the pepsinogen I/II ratio among the three groups (Table 1).

T_{max} value in PPI-resistant NERD patients

We then used the ^{13}C -acetate breath test to calculate the T_{max} value as a marker of gastric emptying of liquids and compared gastric motility among PPI-resistant NERD patients and healthy volunteers. The T_{max} value in PPI-resistant NERD patients was significantly higher than that in healthy volunteers (Fig. 2).

Mosapride citrate combined with omeprazole improves reflux symptoms and T_{max} value in PPI-resistant NERD patients with delayed gastric emptying

To elucidate the possibility that the prokinetic effect of mosapride citrate improved gastro-esophageal reflux symptoms via its effect on gastric emptying, we measured the T_{max} value and clarified whether administration of mosapride citrate in addition to omeprazole affected gastric emptying in PPI-resistant NERD patients with or without delayed gastric emptying. Mosapride citrate in combination with omeprazole therapy significantly improved the T_{max} value in PPI-resistant NERD patients with delayed gastric emptying (Fig. 3). Combined therapy significantly improved T_{max} value in 83% (20/24) of PPI-resistant NERD patients with delayed gastric emptying. We also investigated whether mosapride citrate combined with omeprazole improved the upper GI symptoms and lower GI symptoms. Mosapride citrate combined with omeprazole significantly improved reflux symptoms in the $T_{\text{max}} > 65$ min group of NERD patients compared to that in the $T_{\text{max}} < 65$ min group (Fig. 4).

In some $T_{\text{max}} > 65$ min NERD patients (*n* = 9), we investigated whether combined mosapride citrate and omeprazole treatment affects the acid exposure time and the frequency of acid reflux using 24-h pH monitoring.

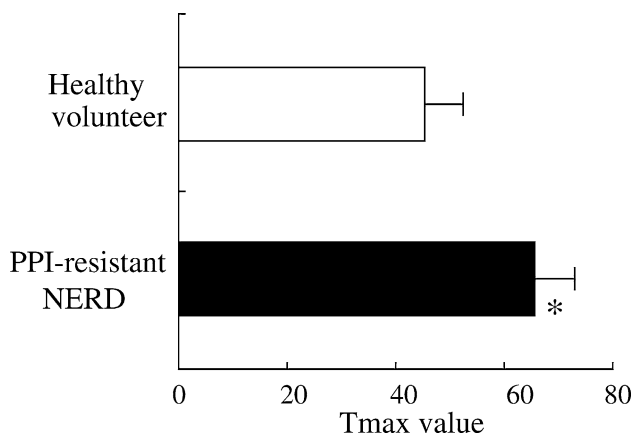


Fig. 2 T_{max} value in PPI-resistant NERD patients and healthy volunteers. The T_{max} value in PPI-resistant NERD patients was significantly higher than in healthy volunteers. *Versus healthy volunteers, $P < 0.05$

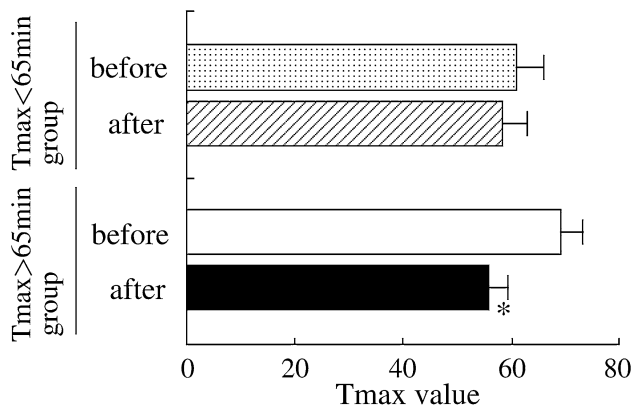


Fig. 3 Mosapride citrate combined with omeprazole improves T_{max} value in PPI-resistant NERD patients with delayed gastric emptying. Mosapride citrate significantly improved ($P < 0.05$) the T_{max} value in $T_{max} > 65 \text{ min}$ NERD patients. *Versus T_{max} value in $T_{max} > 65 \text{ min}$ NERD patients before combined therapy, $P < 0.05$

There was no abnormal acid exposure time ($0.1 \pm 0.08\%$) in nine PPI-resistant NERD patients with delayed gastric emptying under 4-week omeprazole treatment. In addition, there was no difference in the acid exposure time (0.1 ± 0.08 and $0.07 \pm 0.06\%$) and the frequency of acid reflux (5.2 ± 3.2 , 3.6 ± 3.4) in nine PPI-resistant NERD patients with delayed gastric emptying between before and after combined therapy.

Esophageal secondary peristalsis in PPI-resistant NERD patients after combined mosapride citrate and omeprazole therapy

To elucidate the possibility that the prokinetic effect of mosapride citrate improved gastro-esophageal reflux symptoms via its effect on esophageal peristalsis, we measured secondary esophageal peristalsis in NERD

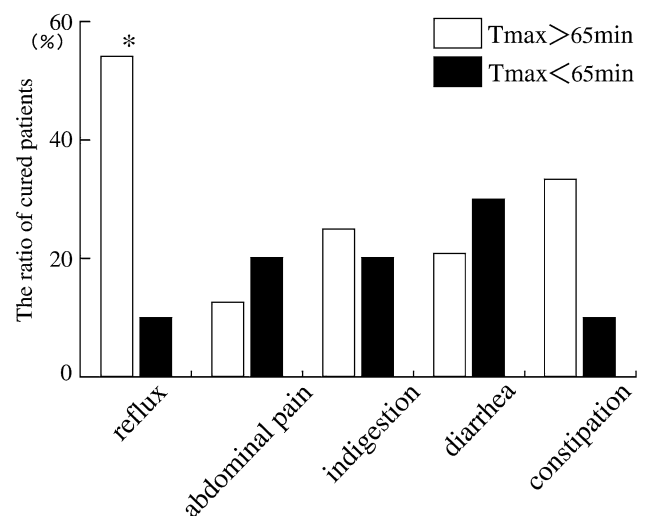


Fig. 4 The ratios of NERD patients with $T_{max} > 65 \text{ min}$ and $T_{max} < 65 \text{ min}$ cured with mosapride citrate and omeprazole co-therapy. Mosapride citrate significantly improved reflux symptoms in PPI-resistant NERD patients with T_{max} value $> 65 \text{ min}$. In contrast, mosapride citrate did not improve any clinical symptoms in PPI-resistant NERD patients with T_{max} value $< 65 \text{ min}$. *Versus the ratio of cured patients after 12 weeks of treatment with mosapride citrate combined with omeprazole in PPI-resistant NERD patients with $T_{max} < 65 \text{ min}$, $P < 0.05$

patients with or without delayed gastric emptying. Although mosapride citrate and omeprazole co-therapy increased the amplitude of primary esophageal peristalsis in some cases (Fig. 5a, b), combined mosapride citrate and omeprazole therapy did not affect primary esophageal peristalsis in most of cases. In addition, mosapride citrate and omeprazole co-therapy did not significantly increase esophageal secondary peristalsis in PPI-resistant NERD patients with or without delayed gastric emptying (Fig. 5c).

Plasma ghrelin levels in PPI-resistant NERD patients after combined mosapride citrate and omeprazole therapy

Mosapride citrate combined with omeprazole did not significantly increase acylated ghrelin levels in PPI-resistant NERD patients with or without delayed gastric emptying (Fig. 6). In contrast, mosapride citrate combined with omeprazole significantly reduced des-acylated ghrelin levels in PPI-resistant NERD patients with delayed gastric emptying (Fig. 6).

Discussion

The major findings in this study are as follows: (1) T_{max} values, one of the markers of gastric emptying, were significantly higher in PPI-resistant NERD patients than in

Fig. 5 Esophageal secondary peristalsis in PPI-resistant NERD patients with or without delayed gastric emptying after combined mosapride citrate and omeprazole therapy. Mosapride citrate and omeprazole co-therapy had no statistically significant effect on esophageal secondary peristalsis in PPI-resistant NERD patients with or without delayed gastric emptying

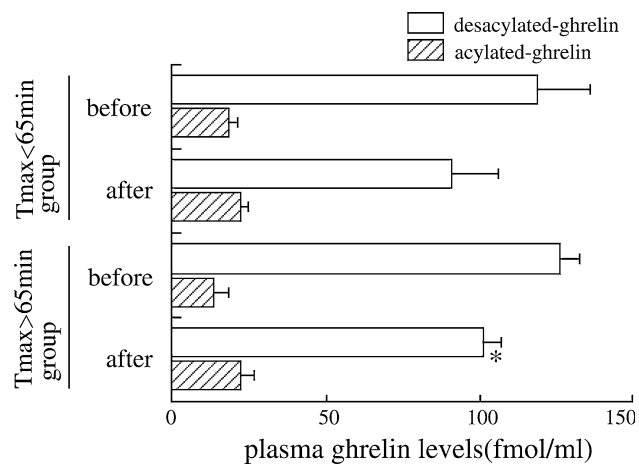
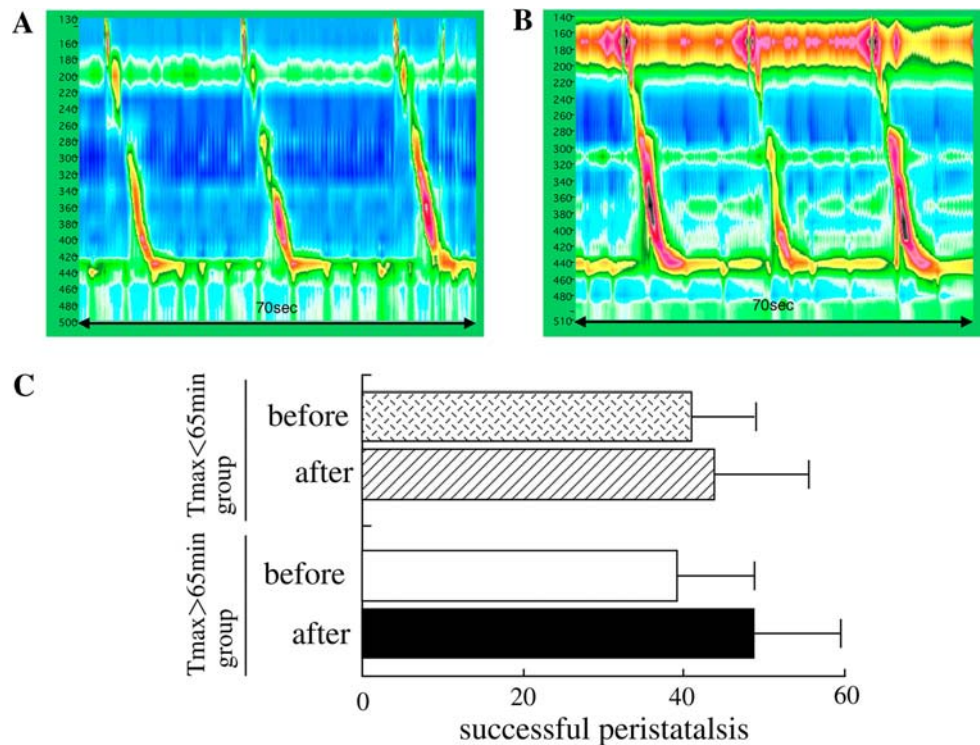


Fig. 6 Mosapride citrate and omeprazole co-therapy reduces desacylated ghrelin levels in PPI-resistant NERD patients with delayed gastric emptying. Mosapride citrate combined with omeprazole significantly reduced the des-acylated ghrelin levels in PPI-resistant NERD patients with delayed gastric emptying. *Versus desacylated-ghrelin levels in $T_{max} > 65 \text{ min}$ NERD patients before combined therapy, $P < 0.05$

healthy volunteers. (2) Administration of mosapride citrate in addition to omeprazole significantly improved reflux symptoms and T_{max} value in PPI-resistant NERD patients with delayed gastric emptying. (3) Mosapride citrate combined with omeprazole significantly reduced des-acylated ghrelin levels in NERD patients with $T_{max} > 65 \text{ min}$.

Non-erosive reflux disease is a chronic disease with high impact on the quality of life [5, 7, 24]. Between 37 and 60% of NERD patients have no increased distal esophageal acid exposure. In addition, they do not respond very well to anti-secretory therapy with PPIs. Several mechanisms have been proposed for the pathogenesis of NERD patients, including visceral hypersensitivity, prolonged contraction of the esophagus and psychological factors [2–4]. We could not find any clinical reports in the available literature regarding the association between gastric emptying and NERD patients. The present study is the first to show that the T_{max} value, which serves as a marker of gastric emptying, was significantly higher in PPI-resistant NERD patients than in healthy volunteers, as confirmed by the ^{13}C breath test. The current standard method for measuring gastric emptying is the radioactive isotope method [25]. The ^{13}C -acetate breath test has also been reported to be a reliable and non-invasive tool for analysis of gastric emptying rates without radiation exposure and to be comparable to scintigraphy [26].

Previous studies have shown that mosapride citrate significantly improved gastric emptying in healthy volunteers, and in patients with Parkinson's disease and diabetic mellitus [16, 27, 28]. Recently, using capsule endoscopy or percutaneous electrogastrogram (EGG), mosapride citrate has been found to improve gastric emptying [29, 30]. We are the first to report that, when combined with omeprazole, the prokinetic effect of mosapride citrate alleviates reflux symptoms and gastric emptying in PPI-resistant NERD patients with delayed gastric emptying, determined

by the ^{13}C -acetate breath test. Some studies have failed to find a relationship between delayed gastric emptying and the pattern of symptoms in FD patients [31, 32]. Quigley et al. [33] have reported that the precise prevalence of delayed gastric emptying remains to be defined in GERD. Ruth et al. [34] have also reported that mosapride citrate decreases acid reflux to the esophagus in GERD patients by its improvement of gastric emptying, as seen in healthy volunteers and diabetic patients for both solids and liquids [16]. Further studies are needed to clarify whether the synergistic effect of mosapride citrate and omeprazole also improves clinical symptoms through their combined effect on gastric motility in PPI-resistant NERD patients.

The current concepts in the pathophysiology of NERD include peripheral factors in the esophageal lumen, such as non-acid reflux, gas reflux and proximal distention of the esophagus [35, 36]. Other studies have shown that roughly 40% of patients with heartburn had normal test results during 24-h intraesophageal pH monitoring [37, 38]. In addition, esophageal hypersensitivity to normal levels of intraesophageal acid may play an important role in the pathogenesis of heartburn, and this hypersensitivity appears to be enhanced in NERD patients [39]. In our study, there was no abnormal acid exposure time in omeprazole-treated NERD patients. Then, after mosapride and omeprazole combined therapy, we could find no significant difference in the acid exposure time and the frequency of acid reflux in PPI-resistant NERD patients in spite of improvements in delayed gastric emptying. A previous study has reported that mosapride citrate inhibited the visceromotor response by gastric distention in conscious rats [40]. Therefore, mosapride and omeprazole cotherapy may act through its modification of visceral sensation. Previous studies have reported that non-acid reflux was also related to symptoms in NERD patients using impedance test [41, 42]. Thus, in addition to more in-depth analysis of esophageal hypersensitivity, further studies are needed to clarify the precise relationship between the acceleration of gastric emptying induced by mosapride citrate and reduction in the positive symptom index for non-acid reflux in PPI-resistant NERD patients with delayed gastric emptying, as measured by impedance test. Esophageal secondary peristalsis is a component of the acid clearance mechanism [43]. Our group has reported that the triggering of secondary peristalsis was disturbed in NERD patients [44]. Ruth et al. [45] have reported that mosapride citrate improved esophageal motor function in GERD patients. Therefore, in this study, we investigated the effect of mosapride citrate on esophageal secondary peristalsis in omeprazole-treated NERD patients with or without delayed gastric emptying. However, we confirmed that mosapride citrate and omeprazole co-therapy did not significantly improve esophageal secondary peristalsis in this group of patients.

In human studies, ghrelin infusion also increased food intake and sensation of hunger as compared with saline infusion alone [46]. Although we investigated whether the prokinetic effect of mosapride citrate improved gastric emptying via the up-regulation of plasma acylated ghrelin levels, mosapride did not significantly affect plasma acylated ghrelin levels in PPI-resistant NERD patients. A previous study has demonstrated that mosapride citrate increases acetylcholine release from parasympathetic nerve endings [14]. Furthermore, Broglio et al. [47] have reported that acetylcholine regulates ghrelin secretion. Therefore, mosapride citrate may increase the T_{\max} value via the up-regulation of acetylcholine-stimulated ghrelin levels. However, in our study, although mosapride treatment improved T_{\max} values in PPI-resistant NERD patients, there was no corresponding elevation in acylated ghrelin levels. In contrast, when combined with omeprazole, mosapride citrate significantly reduced des-acylated ghrelin levels in PPI-resistant NERD patients with delayed gastric emptying. Further studies are needed to clarify the effects of mosapride citrate on des-acylated ghrelin levels. In addition, another study showed that plasma ghrelin levels correlated with gastric atrophy [48]. Suzuki et al. [49] have reported that plasma ghrelin levels correlated well with levels of serum pepsinogen I/II (PGI/II) ratio and decreased with extent of gastric mucosal atrophy. However, in our study, there was no significant difference in the serum pepsinogen I/II ratio between the mosapride-treated group and the non-treated groups.

Taken together, in this study, the prokinetic effect of mosapride citrate combined with omeprazole improved gastro-esophageal reflux symptoms and gastric emptying in PPI-resistant NERD patients with delayed gastric emptying. Further studies are needed to clarify the mechanisms by which mosapride citrate affects gastric emptying in PPI-resistant NERD patients.

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Conflict of interest statement There is no conflict of interest statement in this study.

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