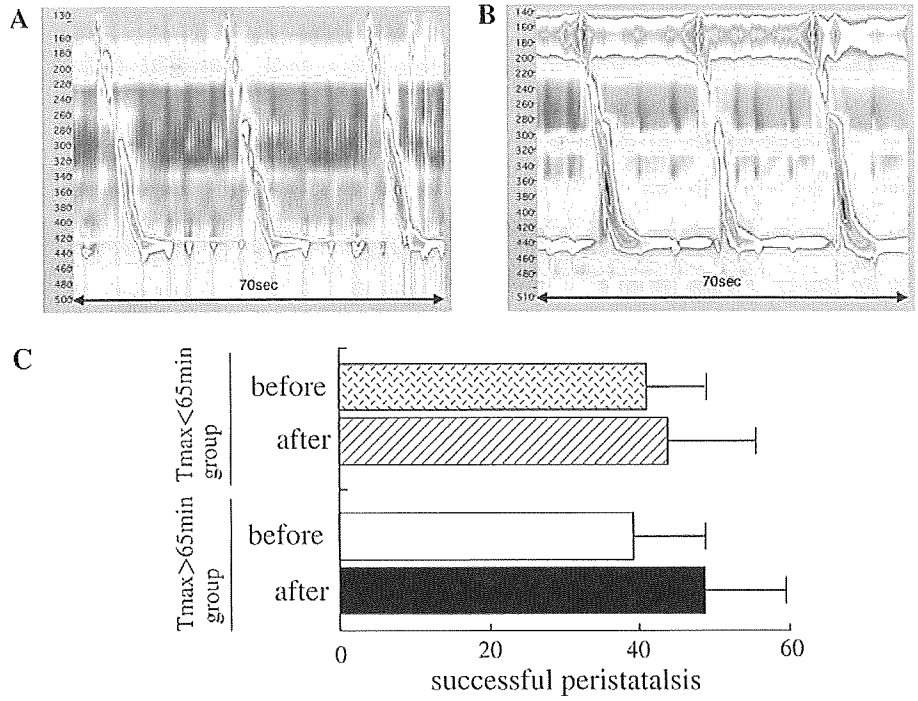

Fig. 5 Esophageal secondary peristalsis in PPI-resistant NERD patients with or without delayed gastric emptying after combined mosapride citrate and omeprazole therapy. Mosapride citrate and omeprazole co-therapy had no statistically significant effect on esophageal secondary peristalsis in PPI-resistant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

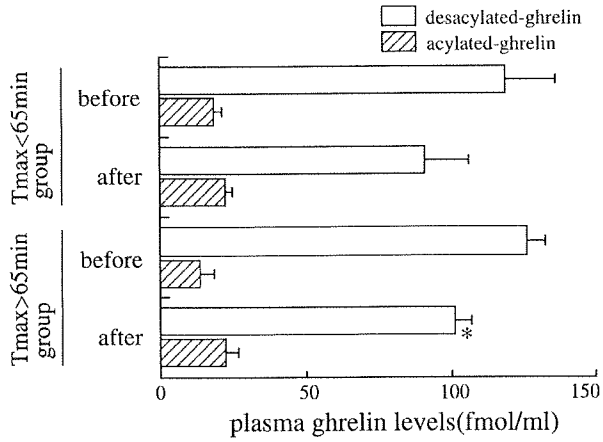

Fig. 6 Mosapride citrate and omeprazole co-therapy reduces des-acylated ghrelin levels in PPI-resistant NERD patients with delayed gastric emptying. Mosapride citrate combined with omeprazole significantly reduced the des-acylated ghrelin levels in PPI-resistant NERD patients with delayed gastric emptying. *Versus desacylated-ghrelin levels in $T_{max} > 65$ 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 $T_{max} > 65$ 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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Duodenogastric reflux induced by endoscopic submucosal dissection

Authors: K. Miyake, M. Kusunoki, T. Shindo, N. Ueki, T. Kawagoe, K. Gudis, A. Tatsuguchi, S. Futagami, T. Tsukui, C. Sakamoto

Institutions: Department of Internal Medicine, Division of Gastroenterology, Nippon Medical School, Tokyo, Japan

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Corresponding author
K. Miyake, MD PhD
Department of Internal Medicine, Division of Gastroenterology
Nippon Medical School
1-1-5 Sendagi, Bunkyo-ku,
Tokyo 113-8603
Japan
Fax: +81-3-56851793
km366@nms.ac.jp

Please see the accompanying editorial by J. J. G. H. M. Bergman on page 988.

Background and study aims: Endoscopic submucosal dissection (ESD) may cause excessive duodenogastric reflux (DGR) in a similar manner to distal gastrectomy, particularly after antral resections. We aimed to examine the occurrence of DGR after ESD.

Patients and methods: Patients with gastric neoplasm for whom ESD was indicated were categorized according to lesion site: the antral group (lower [L] stomach, n = 46) and the nonantral group (upper or middle [U or M] stomach, n = 49). Endoscopy was performed before ESD, the day after ESD, and 3 months after ESD, and the fasting bile acid concentration (BAC) in the gastric juice was analyzed.

Results: BAC values showed significant interaction between time point and group, although this association differed in the antral and nonantral groups. BACs on the day after ESD were higher in the antral group than in the nonantral group, but not the pre-ESD and 3 months post-ESD levels. In the antral group only, fasting BACs increased significantly the day after ESD and decreased to baseline levels 3 months post-ESD. There was also a correlation between BAC and lesion location in the antral subgroups, with significantly higher BACs found the day after ESD in patients with lesser curvature lesions.

Conclusions: ESD of lesions in the antral lesser curvature may lead to a transient early increase in DGR. However, ESD does not result in long-term DGR, a factor that is known to increase the risk of carcinogenesis following gastrectomy.

Introduction

Epidemiological studies have shown that patients who undergo distal gastrectomy are at increased risk of developing gastric remnant carcinoma. Numerous factors influence such a development, including hypochlorhydria, bacterial overgrowth, hypogastrinemia, and duodenogastric reflux (DGR). The incidence of remnant carcinoma is lower in post-Billroth I compared with post-Billroth II gastrectomy, since the latter usually results in higher DGR in the remnant stomach for anatomical reasons [1–5]. In addition, most remnant carcinomas are located near the anastomosis [6,7]. This evidence implicates prolonged exposure to DGR as a major risk factor for remnant carcinoma, following reflux gastritis or a precancerous state. Furthermore, DGR is reportedly associated with development of dyspeptic symptoms, Barrett's metaplasia and dysplasia [8–17]. Based on such findings, surgical reconstruction procedures such as Roux-en-Y and jejunal interposition have been attempted to prevent DGR [18–20]. However, Roux-en-Y reconstruction often results in Roux-en-Y syndrome, which is characterized by symptoms of upper gut stasis such as abdominal pain, nausea, and vomiting postoperatively [21,22]. Given this background, DGR is considered to be the most frequent and problematic complication after distal gastrectomy.

Endoscopic mucosal resection (EMR) has been widely applied as a less invasive technique for the removal of intramucosal neoplasms in the gastrointestinal tract. Accumulation of data from surgically treated gastric cancers indicates that a certain group of early cancers with an extremely low risk of lymph node metastasis can be treated endoscopically [23]. En bloc resection is the ideal option, but conventional EMR using excisional snaring cannot provide this for larger lesions [24–29], and endoscopic submucosal dissection (ESD) was developed to overcome the technical limitations of EMR [20,30–32]. ESD permits en bloc resection for larger lesions and provides a higher rate of histologically complete resection (> 80%) compared with conventional EMR [33]. However, ESD is technically more complex and time-consuming, and may also cause morpholo-

gical and functional impairment of the stomach which may manifest as hypoperistalsis, a burning sensation of the muscularis propria or nerves, and as deformation of the stomach by extensive artificial ulceration [20,32–34]. Therefore, as with distal gastrectomy, ESD may cause excessive DGR, particularly after procedures on the antral area, which is thought to play an important role in the control of DGR. However, the association between ESD and the development of DGR has not been clarified. Therefore, the present study was carried out to evaluate whether ESD for gastric neoplasm induces DGR as a short-term and/or long-term outcome, and whether this is dependent on the resection site.

Patients and methods

Participants

The participants were patients aged 20 to 85 years old with gastric neoplasms for which ESD was indicated, based on the judgment of an extremely low risk of lymph node metastasis from diagnosis using magnified chromoendoscopy and pathological evaluation of biopsies. Patients with synchronous or metachronous gastric cancer, major organ failure, or history of gastrectomy or reconstructive surgery of the stomach tube for treatment of esophageal cancer were excluded. Those who did not provide written informed consent were also excluded from the study.

The lesions were characterized endoscopically according to the Paris classification [35], and microscopic histological classification was done according to the revised Vienna classification of gastrointestinal epithelial neoplasia [36,37]. The location of the lesion was described using the UML classification, where the lesser and greater curvatures are divided into three equal lengths: U, upper; M, middle; and L, lower [38]. The ESD patients were divided into two groups according to the UML classification of their lesions: the antral group (lesions mainly located in the L region); and the nonantral group (lesions mainly located in the U or M region).

Omeprazole (20 mg) was administered intravenously 2 h before ESD, 12 h after the first dose, and twice on the second day. From the third day after ESD, omeprazole was administered orally for 8 weeks, followed by administration of histamine H_2-receptor antagonists until endoscopic examination at 3 months post-ESD.

This study was approved by the Clinical Research Ethics Committee of the Nippon Medical School.

ESD procedure

The ESD procedure for gastric neoplasm was performed using a flex knife (KD-630L; Olympus Medical Systems, Tokyo, Japan) and/or an insulation-tipped knife (KD-610L; Olympus) with an ICC-200 or VIO300D electrosurgical generator (ERBE, Tübingen, Germany), after injection of 10% glycerine solution with a small amount of epinephrine and indigo carmine. The artificial ulcer was carefully examined after resection and any visible vessels were coagulated by argon plasma coagulation or hot biopsy forceps using the soft coagulation mode (80 W). The dissection field during ESD was repeatedly flushed with an adequate amount of water to preserve the endoscopic view, and all accumulated fluid in the stomach was suctioned at completion of the procedure.

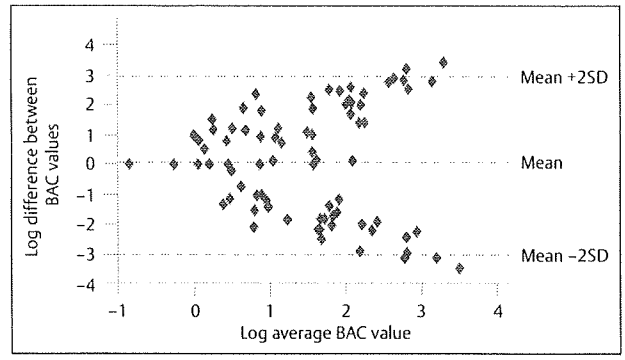

Fig. 1 Bland-Altman plots of log-transformed data were used to analyze reliability and intraindividual variation for measurements of fasting bile acid concentration (BAC). Two measurements were taken on separate days in 82 patients.

Measurement of fasting bile acid concentration (BAC) in gastric juice

After an overnight fast, patients were treated with 2% xylocaine spray, which was sufficient to anesthetize the hypopharynx, and upper endoscopy was performed to obtain gastric juice samples. DGR was assessed on the basis of the fasting BAC level in gastric juice [39–42], which was measured by the following modified method, as a scarcity of the gastric juice needed for BAC measurement could have impaired the reliability and reproducibility of our DGR evaluation. Therefore, to ensure as thorough an examination of DGR events as possible, refluxed bile that adhered to the mucosa was washed out endoscopically from the antrum through the stomach with 20 ml of spray water. Using this procedure, the total amount of fluid in the stomach was collected endoscopically at three time points: before ESD, on the day after ESD, and 3 months post ESD. For each sample, an aliquot of gastric juice (≥ 5 ml) was centrifuged for 5 min at 2000 rpm and then the supernatant was stored at -20 °C for analysis within a month. The fasting BAC in the sample was measured using enzymatic spectrocolorimetry (Enzabile; Nycomed Pharma AS, Oslo, Norway).

To establish the reliability of the method, Bland-Altman plots with log-transformed data were used to analyze reliability and intraindividual variation between two measurements of fasting bile acid on separate days, in 82 patients. These were patients with chronic gastritis, aged 20 to 85 years. Patients with synchronous or metachronous gastric cancer, major organ failure, history of gastrectomy, medication with proton pump inhibitors (PPIs), or reconstructive surgery of the stomach tube for treatment of esophageal cancer were excluded, as were those who did not provide written informed consent. As shown in ◉ **Fig. 1**, although the difference for each pair of the 82 sets of measurements tended to increase with the mean of the two measurements, the coefficient of repeatability (2 × SD) was 1104.2 µmol/L and 95.1% (78/82) of the differences fell within 2 SD.

Statistical analysis

Depending on the type of baseline data, the results for the two groups were compared using a paired or unpaired Student *t* test for continuous variables, a Fisher exact test for categorical data and the Bonferroni–Dunn test for multiple comparisons. Continuous variables are expressed as means and standard error (SE). Repeated-measures one-way analysis of variance (ANOVA) was performed on the BAC data to identify interactions between

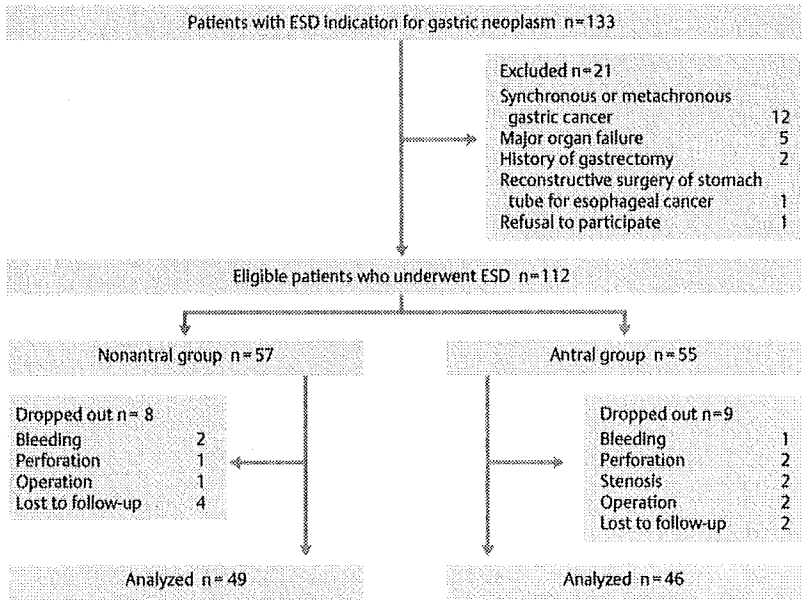

Fig. 2 Progress of patients in the study.

time and groups, or time-specific effects for each group. Differences with two-tailed P values < 0.05 were regarded as statistically significant.

Results

Recruitment and patient characteristics

From February 2005 to September 2007 at our hospital, a total of 133 patients with gastric neoplasms had indications for ESD. Of these, 21 were excluded due to synchronous or metachronous gastric cancer (n = 12), major organ failure (n = 5), history of gastrectomy (n = 2) or history of reconstructive surgery of the stomach tube for esophageal cancer (n = 1). Thus, 112 patients underwent ESD (**Fig. 2**).

Fasting BA concentrations in gastric juice could not be estimated in 8 patients in the nonantral group (active bleeding the day after ESD, 2; development of complicating gastric perforation requiring nasogastric suction, 1; additional gastrectomy for invasive submucosal cancer [submucosal invasion > 500 µm, sm2], 1; or loss to follow-up, 4), and in 9 patients in the antral group (active bleeding the day after ESD,1; complicating gastric perforation requiring nasogastric suction, 2; stenosis requiring balloon dilation, 2; additional gastrectomy for invasive submucosal cancer [sm2], 2; or loss to follow-up, 2).

Therefore, 95 patients (66 men, 29 women; mean age, 70.5 ± 1.2 years; antral group, n = 46; nonantral group, n = 49) who were followed for > 3 months after ESD were included in the analysis. Baseline characteristics did not differ significantly between the two groups, except for a higher rate of histopathological adenocarcinoma and a longer resection time in the nonantral group (each $P < 0.05$) (**Table 1**).

Influence of ESD on the fasting BAC in gastric juice

Significant time-specific effects were found for the BAC levels of the patients overall ($P = 0.028$ by repeated-measures one-way ANOVA). The fasting BAC in gastric juice collected the day after ESD tended to be elevated compared with the pre-ESD value (721.0 ± 178.6 vs. 380.3 ± 122.5 µmol/L, not a significant difference by paired Student t test). The fasting BAC in samples collected 3–6 months post ESD was significantly lower than that in samples collected the day after ESD (239.5 ± 60.0 vs. 721.0 ± 178.6 µmol/L; $P < 0.01$ by paired Student t test). The fasting BAC in samples collected before ESD and at 3 months post ESD did not differ significantly.

After interactions for times and groups were statistically confirmed ($P = 0.008$ by repeated-measures one-way ANOVA), the BAC levels of the antral and nonantral groups. were compared at each time point. The BAC levels showed a higher level in the antral group than in the nonantral group at the day after ESD (1132.8 ± 346.2 vs. 334.4 ± 96.0 µmol/L; $P < 0.025$, unpaired Student t test), but not at baseline (pre-ESD) or 3 months after ESD (**Fig. 3**).

After time-specific effects were found by repeated-measures one-way ANOVA in the antral group ($P = 0.004$) but not in the nonantral group, the BAC levels between the time points in the antral group were compared. In the antral group, the fasting BAC in gastric juice was significantly higher the day after ESD compared with pre-ESD (1132.8 ± 346.2 vs. 292.8 ± 117.3 µmol/L,

Table 1 Resection of gastric neoplasia by endoscopic submucosal dissection (ESD): clinicopathological characteristics in patients with antral and nonantral lesions.

	Antral	Nonantral	P value
Patients, n	46	49	
Age, mean ± SE, years	70.9 ± 1.2	70.2 ± 1.2	n. s.
Male : female	34:12	32:17	n. s.
Smoking, yes : no	15:31	15:34	n. s.
Resection time, mean ± SE, min	129.5 ± 8.9	163.9 ± 12.4	0.028
Resected specimen size, mean ± SE, mm	36.6 ± 1.9	35.3 ± 1.6	n. s.
Histopathology, n (%)			
Adenoma, low grade	5 (10.9)	1 (2.0)	n. s.
Adenoma, high grade	16 (34.8)	10 (20.4)	n. s.
Adenocarcinoma	25 (54.3)	38 (77.6)	0.017
Complete resection rate	43 (93.5)	39 (79.6)	0.0497

$P = 0.022$, paired Student t test, ○ **Fig. 3**), and significantly lower at 3 – 6 months post ESD compared with the day after ESD (202.7 ± 52.4 vs. 1132.8 ± 346.2 µmol/L; $P = 0.008$, paired Student t test, ○ **Fig. 3**). There was no significant difference between fasting BACs in pre-ESD samples and those collected at 3 months post ESD.

Detailed relationship of antral resection sites and fasting BAC

To clarify in more detail the relation between antral locations and elevation of BAC post ESD, we examined the longitudinal location of the resection area with regard to the increase in BAC from before ESD to the day after ESD, in the antral group (n = 46). The group was divided into "prepyloric" (n = 11) and "nonprepyloric" (n = 35) subgroups, in whom the distance from the distal margin of the tumor to the pyloric ring was < 1 cm and ≥ 1 cm, respectively. This characteristic was chosen because ulceration due to ESD at a prepyloric site may directly affect the function of the pyloric ring. However, unexpectedly, one-way ANOVA showed no significant interactions between time point and group for the BAC levels for the two subgroups (○ **Table 2**).

Next, we delineated the axial location of lesions in the antral group and examined four subgroups based on the predominant location of the lesion in the antrum: the posterior wall (n = 12); the lesser curvature (n = 8); the anterior wall (n = 16); and the greater curvature (n = 10). Statistically significant interactions between time and group were found for these subgroups ($P = 0.005$, one-way ANOVA). Comparisons among the subgroups revealed higher BAC levels for lesions in the lesser curvature than in the other subgroups on the day after ESD (3624.6 ± 1419.1 [lesser curvature] vs. 465.3 ± 195.5, 473.7 ± 261.7 and 407.0 ± 198.9 µmol/L in the anterior wall, greater curvature, and posterior wall subgroups, respectively; $P < 0.01$, unpaired Student t test; ○ **Fig. 4**), but not for pre-ESD values.

Significant time-specific effects were also found in the lesser curvature subgroup ($P = 0.005$ by repeated-measures one-way ANOVA), but not in the other subgroups. In the lesser curvature subgroup, the fasting BAC level in gastric juice collected the day after ESD was significantly higher than that in gastric juice collected before ESD (3624.6 ± 1419.1 vs. 264.1 ± 154.8 µmol/L; $P < 0.05$, unpaired Student t test; ○ **Fig. 4**).

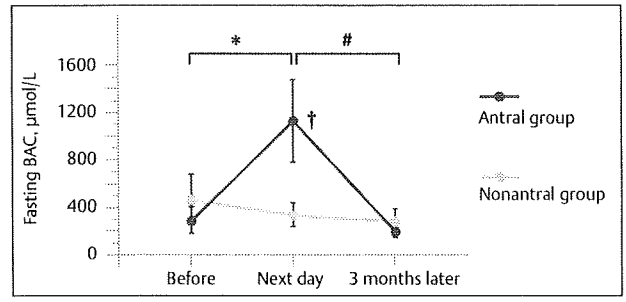

Fig. 3 Endoscopic submucosal dissection (ESD) of gastric neoplasms and the fasting bile acid concentration (BAC) in gastric juice in patient groups with antral and nonantral lesions. Comparing the antral and nonantral groups, a higher BAC level was seen in the antral group († $P < 0.025$) the day after ESD, but not at baseline (pre-ESD) or 3 months after ESD. Within the antral group, the fasting BAC was significantly higher on the day after ESD compared with before ESD (* $P = 0.022$), and significantly lower at 3 – 6 months post ESD compared with the day after ESD (# $P = 0.008$).

Discussion

ESD, which unlike conventional EMR permits en bloc resection of larger lesions, induces stress that may lead to extensive artificial ulceration in the stomach [20, 32 – 34], or to morphological and functional impairment of the stomach which may cause DGR; this is particularly the case with ESD of antral lesions. The purpose of the present study was to evaluate whether ESD for gastric neoplasms induces DGR in the short or long term, based on the measurement of fasting BACs in gastric juice collected endoscopically at three predetermined time points: before ESD, on the day after ESD, and 3 months post ESD. As expected, patients in the antral group showed significant aggravation of DGR on the day after ESD, although the effect was transient. No aggravation of DGR occurred in the nonantral group. Thus, based on the absence of significant elevation of BAC levels at 3 months post ESD compared with the pre-ESD levels, neither antral nor nonantral lesions were associated with long-term DGR. This is the first systematic report assessing the possible influence of ESD on DGR following the endoscopic intervention.

There is no gold standard for the estimation of DGR and several methods have been used for its evaluation, including ambulatory pH monitoring, bilirubin monitoring, scintigraphy, determination of bile acids or bilirubin in aspirated gastric juice, and histology. Several clinical studies have used bile acid concentrations in gastric juice to assess the extent of DGR [39 – 42], and studies in vitro have shown good correlation between ambulatory "Bilitec" measurements and bile acid concentrations [43 – 45]. The most important aim of the present study was to verify whether DGR, which may affect post-ESD ulcer healing or cause complications, was aggravated during the early stage after ESD; this is a time when the ulcer is fragile and could easily bleed, since 76% of bleeding induced by ESD is seen within 24 hours after dissection [46]. Thus, we thought the best approach for this study would be to measure fasting BAC, as this could be accomplished with minimal stress for patients during routine endoscopic examination. However, we understand that the absence of a gold standard for the evaluation of DGR could hinder validation of the method used in this study.

It also became clear, early in the preparatory stages of this study, that the conventional method for measuring BACs could not be used in cases where no gastric juice production could be detected. This lack of gastric juice production, due to atrophy of the corpus or the administration of PPIs or histamine H_2-receptor antagonists, could impair both the reliability and reproducibility of our evaluation of DGR because it might not always be possible

Table 2 Bile acid concentration (BAC) before and after endoscopic submucosal dissection (ESD), related to antral site: prepyloric (n = 11) and nonprepyloric (n = 35), where the distance between the distal margin of the tumor and the pyloric ring was < 1 cm and ≥ 1 cm, respectively. Unexpectedly, no significant interaction between time point and site was found by one-way analysis of variance (ANOVA).

	Bile acid concentration (BAC), µmol/L	
	Before ESD	Day after ESD
Antral site		
Prepyloric site	737.3 ± 415.9	1950 ± 1024.6
Nonprepyloric site	153.1 ± 74.4	875.8 ± 321.5

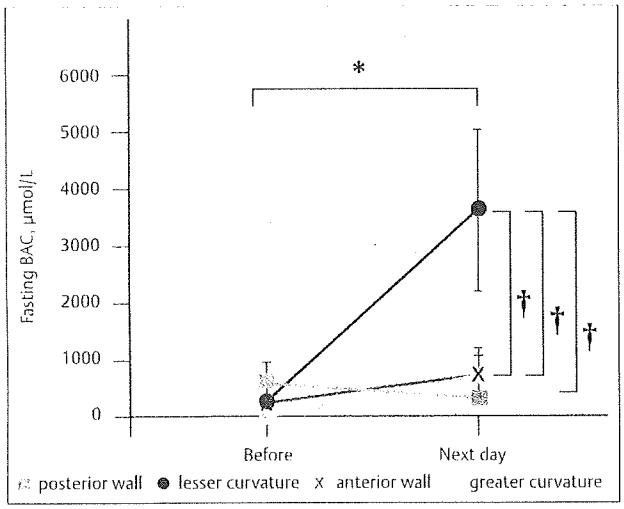

Fig. 4 Fasting bile acid concentration (BAC) in gastric juice collected endoscopically before ESD and on the day after ESD from patients in the antral group, according to axial location of the lesion (lesser curvature, greater curvature, posterior wall anterior wall). Comparison revealed higher BAC levels for lesions in the lesser curvature than in the other subgroups for the day after ESD (†$P < 0.01$, for each), but not for before ESD. Significant time-specific effects were also found in the lesser curvature subgroup ($P = 0.005$, by repeated-measures one-way ANOVA), but not in the other subgroups; in this subgroup, the fasting BAC level the day after ESD was significantly higher than that in gastric juice collected pre-ESD (*$P < 0.05$, by unpaired Student t test).

perform all three necessary measurements: before ESD, on the day after ESD, and 3 months post ESD. Therefore, to ensure that our evaluation of DGR was as thorough as possible, we sprayed the mucosa from the antrum through the stomach with 20 ml of rinse water to wash out any adhering refluxed bile. Using this procedure, the total amount of fluid in the stomach was collected endoscopically at the three time points.

However, this method required biochemical validation in the absence of a gold standard for estimation of DGR. Therefore, we performed two measurements on separate days for 82 patients without active peptic ulcer or gastric cancer, and obtained a correlation coefficient that indicated that the method was reliable for measurement of BAC in the gastric juice. Bland-Altman plots of log-transformed data were used to analyze the reliability of this spraying solution method and its effect on intraindividual variation between two measurements of fasting bile acid on separate days in the 82 patients. As shown in **Fig. 1**, although the difference for each of the 82 pairs of values tended to increase with the mean value of the two measurements, the coefficient of repeatability (2 × SD) was 1104.2 μmol/L, and 95.1% (78/82) of the differences fell within 2 SD. Thus, we concluded that our method of measuring fasting bile acid was reliable and that intraindividual variations would not be significant. Further, although the reduction of gastric fluid volume due to the administration of PPIs or histamine H_2-receptor antagonists can affect gastric BACs, the intraindividual variation could be minimized by serial measurement, and the administration conditions could be met at the three determined time points for each patient.

The mechanisms underlying the transient increases in fasting DGR at 1 day after ESD in the antral group are unclear, but may result from factors including an open pylorus, motility changes, vagal disturbances, and other pathophysiologic features. We initially speculated that ESD procedures on prepyloric lesions in the antral group would predominantly influence DGR by impairing pyloric motor function, which plays an important role in gastric emptying and DGR prevention [47–49]. However, the division of antral lesions into prepyloric and nonprepyloric sites based on the distance of the tumor from the pyloric ring, and subsequent analysis by one-way ANOVA, did not show significant interactions between time and group for these two subgroups. Therefore, we evaluated the effect of lesion location on increased DGR by dividing the antral group into subgroups according to location in the posterior wall, lesser curvature, anterior wall, or greater curvature. Although the sample sizes of each subgroup may be inadequate, interestingly, the increase in fasting BAC in the antral group the day after ESD was significantly larger for lesions in the lesser curvature; which could indicate that the pathophysiologic mechanisms underlying DGR after ESD are associated with a specific deformity of the pyloric ring in the lesser curvature or a disturbance of the vagus nerve, which is predominant in the lesser curvature region.

DGR has a normal physiological role to a certain extent [39,50,51], but reflux of bile and duodenal contents, i.e., pancreatic enzymes, is a well-known major pathological factor in reflux gastritis. These contents exert pathological effects through various mechanisms, including increased mucosal permeability to acid and direct damage to surface cells of the stomach, which render the gastric mucosa more susceptible to acid injury [52–54]. A BAC > 1000 μmol/L is considered abnormal and indicative of clinically important DGR [40], and Sobala et al. have shown that only 1 in 50 patients who had not been treated surgically had a BAC > 1000 μmol/L in gastric juice [41]. In addition, the mean postprandial BAC in patients with gastric ulcer is reportedly higher than the fasting BAC levels, using point measurements of BAC as in our study [40]. Therefore, the stomach milieu resulting in evident DGR in the early period after ESD is likely to be more severe than anticipated and may involve pathological conditions that can exert deleterious effects.

The risk of bleeding during ESD is thought to be higher in lesions of the upper stomach region, which contains abundant blood vessels and is a technically problematic site. However, the incidence of bleeding at 0–30 days after ESD (so-called delayed bleeding) is reportedly higher in lesions of the middle and lower stomach [55]. The high frequency of bleeding from ESD-related artificial ulcers in the lower portions of the stomach may result from insufficient intraoperative hemostatic treatment of vessels, based on the anticipated lower risk of bleeding compared with lesions in the upper third of the stomach. Transient aggravation of DGR in the early period after ESD could have adverse affects on the bleeding stability and healing of artificial ulcers, and this may be one of the causes of the high frequency of bleeding from artificial ulcers in the lower portion of the stomach in the antral group. Further studies are required to clarify the precise mechanism of DGR.

Distal gastrectomy is the established standard operative procedure for the treatment of gastric cancer. However, dysplasia or carcinoma in gastric remnants, and Barrett's metaplasia and dysplasia, can develop postoperatively through mucosal cell damage or acceleration of cell proliferation in gastric and esophageal mucosa, due to exposure to bile acid or duodenopancreatic juice [8–17]. ESD resulting in long-term DGR may also be implicated as a causal factor in heterochronia, but our results suggest that ESD is not associated with carcinogenesis via long-term DGR, in contrast to distal gastrectomy.

A spectrum of surgical techniques are available for the treatment of gastric cancer, ranging from open total or subtotal gastrectomy with lymph node dissection to minimally invasive procedures such as laparoscopic gastrectomy. Laparoscopy-assisted gastrectomy offers several advantages, including better cosmetic effects, reduced blood loss, less impairment of nutrition, reduced pain, more rapid gastrointestinal functional recovery, and a shorter duration of hospitalization, without a decrease in operative efficacy compared with conventional open gastrectomy [56–61]. These advantages are particularly attributed to laparoscopy-assisted distal gastrectomy (LADG), and the use of LADG procedures has dramatically increased, and largely replaced conventional open gastrectomy, particularly in Japan [62]. In addition, LADG avoids the greatest shortcoming of ESD by permitting lymph node dissection. As a less invasive gastrectomy procedure, LADG may thus offer a powerful alternative for the treatment of early gastric cancers in the lower or middle stomach, even when the lesion meets the general indications for ESD. Nevertheless, considering the long-term complications involved in the development of dysplasia or carcinoma in the remnant stomach after distal gastrectomy, our evidence that long-term DGR is not induced by ESD suggests a distinct advantage for ESD as the therapy of choice, provided that the risk of lymph node metastasis is extremely low.

In summary, our results indicate that ESD for treating antral lesions at the lesser curvature leads to a transient increase in DGR immediately after the procedure. In the long term, ESD is unlikely to cause reflux gastritis or carcinogenesis by inducing DGR, in contrast to distal gastrectomy. However, deleterious contents in DGR may influence the ulcer-healing processes in the early stage after ESD for antral lesions, and this aspect of the procedure requires examination in greater detail.

Competing interests: None of the authors have any conflicts of interest to disclose.

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