Table 4 Association of gastric corpus atrophy and gallstones with the presence of US-ESEM or SS-ESEM

	US-ESEM			SS-ESEM		
	ESEM(+) No./total no. (%)	ESEM(−) No./total no. (%)	Odds ratio (95% CI)[c]	ESEM(+) No./total no. (%)	ESEM(−) No./total no. (%)	Odds ratio (95% CI)[c]
Gastric corpus atrophy (−)[a], gallstones (−)	107/218 (49.1)	139/233 (59.7)	0.61 (0.41–0.89)*	69/143 (48.3)	88/151 (58.3)	0.63 (0.39–1.02)▲
Gastric corpus atrophy (+)[b], gallstones (−)	64/218 (29.4)	51/233 (21.9)	1.56 (1.01–2.40)*	37/143 (25.9)	42/151 (27.8)	0.92 (0.54–1.56)
Gastric corpus atrophy (−)[a], gallstones (+)	27/218 (12.4)	31/233 (13.3)	0.92 (0.53–1.61)	17/143 (11.9)	13/151 (8.6)	1.45 (0.68–3.11)
Gastric corpus atrophy (+)[b], gallstones (+)	20/218 (9.2)	12/233 (5.2)	1.99 (0.94–4.21)▲	20/143 (14.0)	8/151 (5.3)	2.99 (1.26–7.06)*

US-ESEM ultrashort-segment endoscopically suspected esophageal metaplasia, *SS-ESEM* short-segment endoscopically suspected esophageal metaplasia, *CI* confidence interval

▲ $p < 0.1$, *$p < 0.05$

[a] Severity of gastric mucosal atrophy, none or mild

[b] Severity of gastric mucosal atrophy, moderate or severe

[c] Adjustment for age and gender

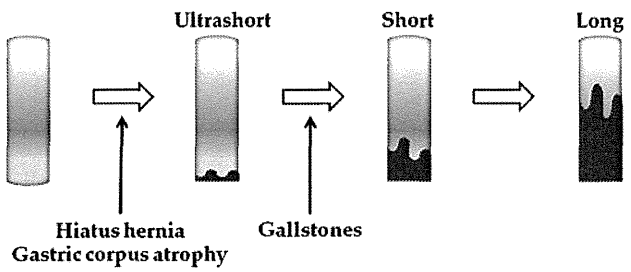

Fig. 2 Risk factors for the elongation of ESEM. The risk factors for US-ESEM and SS-ESEM were different. Hiatus hernia was associated with the presence of both US- and SS-ESEM. Gastric corpus atrophy was associated with the presence of US-ESEM, but its association with the presence of SS-ESEM was not statistically significant. Gallstones were associated with the presence of SS-ESEM, but not with that of US-ESEM

gastric mucosal atrophy was marginally associated with the presence of US-ESEM, but not with that of SS-ESEM. Presence of gastric corpus atrophy without gallstones was significantly associated with the presence of US-ESEM, but not with that of SS-ESEM. On the other hand, the presence of gallstones was significantly associated with the presence of SS-ESEM, but not with that of US-ESEM. The presence of severe reflux esophagitis was also associated with the presence of SS-ESEM, but not with that of US-ESEM (Fig. 2). The presence of *H. pylori* infection was not associated with the presence of either US- or SS-ESEM.

These results suggest that the etiology of US-ESEM may differ from that of SS-ESEM. The presence of gastric corpus atrophy appeared to be a necessary condition for the development of US-ESEM. El-Serag et al. [24] also reported that while USBE was not associated with the presence of erosive esophagitis, it showed a positive association with the presence of gastric mucosal atrophy and metaplasia. Known risk factors for intestinal metaplasia at the gastric cardia include advanced age, male sex, and severe bile reflux [25]. Ye et al. [26] reported that adenocarcinoma of the gastric cardia was not associated with the presence of *H. pylori* infection, but it showed a positive association with the presence of gastric mucosal atrophy, which can be caused by exposure of the stomach to bile. The etiological association of USBE may be similar to that of adenocarcinoma of the gastric cardia, and the condition appears to be caused by duodenogastric reflux of bile into the gastric corpus.

On the other hand, the epidemiology of SS-ESEM appeared to be similar to that of esophageal adenocarcinoma. The presence of gallstones and reflux esophagitis has been reported to be associated with abnormal acid and bile reflux into the esophagus [27, 28]. Akiyama et al. [29] reported that the prevalence of erosive esophagitis was higher in subjects with a circumferential length of Barrett's epithelia of at least 2 cm than in those of less than 2 cm. Okita et al. [30] reported that the severity of reflux esophagitis, reflux symptoms, and hiatus hernia was positively correlated with the length of SSBE. These studies also suggest that the presence of severe gastroesophageal reflux is a necessary condition for the elongation of BE.

The limitation of the present study was potential selection bias due to it being a hospital-based study. Subjects in the present study were older than the general population, and most of them had some diseases. The prevalence of gallstones would also be higher. To verify the results of the

present study, a prospective study based on a health check-up cohort would be needed.

In conclusion, the epidemiology of US-ESEM and SS-ESEM was divergent. In Japan, USBE might account for the majority of patients with BE. US-ESEM was associated with the presence of gastric corpus atrophy, but not with the presence of *H. pylori* infection, suggesting that US-ESEM may be associated with duodenogastric bile reflux, which is also known to be associated with the development of gastric corpus atrophy. On the other hand, SS-ESEM was not associated with the presence of gastric mucosal atrophy, suggesting that a large amount of gastroesophageal acid reflux was necessary for the development of SS-ESEM. Further studies are needed for histological confirmation of our findings.

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Review

Ghrelin and oxidative stress in gastrointestinal tract

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Oxidative stress is a major cause of the gastrointestinal damage under physical or psychological stress. Ghrelin exhibits gastroprotective effects and they are supposed to be derived from antioxidant effects. In gastroduodenal mucosal injury, the plasma ghrelin levels increase in response to the demand for gastroduodenal cytoprotection. However, in the condition of *Helicobacter pylori*-induced gastric mucosal severe atrophy, the plasma ghrelin concentration shifted to lower levels. In diabetic gastroparesis, the regulation of ghrelin secretion is impaired with vagal nerve dysfunction. Selective ghrelin agonist is expected to represent a new class of prokinetic agent. In addition, the plasma ghrelin levels are also enhanced by systemic oxidative stress, and ghrelin exhibits antioxidant effects in many organs, such as heart, pancreas, and lung. This suggests that ghrelin would be an important player as a sensor of systemic oxidative stress.

***Key Words*:** **oxidative stress, ghrelin, peptic ulcer, gastroparesis**

The physiological response to stressor includes an increased activity of the hypothalamic-pituitary-adrenal axis as well as changes in gastrointestinal damage. According to Selye's formulation of the general adaptation syndrome, an increase in adrenocortical activity should be related to an increase in the incidence of gastric ulceration.[1] The strong candidate for the cause of stress ulcer would be oxidative stress. There are some evidences that not only physical stress, such as surgery and infection, but also psychological stress leads to oxidative stress.[2,3]

Oxidative stress, which refers to a state of elevated levels of reactive oxygen species (ROS), forms a variety of conditions that stimulate either ROS production or a decline in antioxidant defenses. Oxidative stress is involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies.[4] Several gastrointestinal diseases, such as peptic ulcer disease and gastroparesis, are known to be related with the dysfunction of the antioxidative properties.[5]

Ghrelin, produced and secreted by the A-like cells of the oxyntic glands of the stomach, stimulates growth hormone (GH) secretion, gastric motility, and food intake.[6] Many researchers reported the relationship between oxidative stress and the expression or function of ghrelin.[7,8] Moreover, ghrelin administration is expected to reduce oxidative stress and control diseases.[9] Previous studies have reported that ghrelin has an anti-inflammatory action on the oxidative injury of the diverse organs, such as the heart, liver and pancreas.[10–14] In the present article, we discuss the association of oxidative gastrointestinal damages with the potential role of ghrelin.

Effects of Ghrelin against Gastric Mucosal Injury

Recent studies have shown that ghrelin exhibits gastroprotective effects.[15–19] Ghrelin administration reduced ethanol-induced gastric ulceration,[15,17,18] acetic acid-induced chronic gastric and duodenal ulceration,[16] and ischemia-reperfusion (I/R)-induced gastric ulceration[18,19] in rats. In addition, ghrelin administration increased mucosal cell proliferation[16] and mucosal microvascular flow[16,18,20] in rats. These effects could be observed by intracerebroventricular,[15,17,18] subcutaneous,[15] intraperitoneal,[18] and peripheral intravascular [19] administration of synthetic ghrelin.

The mechanism of the gastroprotective effects of ghrelin remains unclear. Sibilia *et al.* reported that such effects of ghrelin are mediated by endogenous nitric oxide (NO) release and requires the integrity of sensory nerve fibers.[15] Sibilia *et al.*[17] also reported that cyclooxygenase-1-derived prostaglandins (PGs) are mainly involved in ghrelin-associated gastroprotection and that NO derived from constitutive source, together with PGE_2, are involved in its activity. Ceranowicz *et al.*[16] reported that the gastroprotective effects of ghrelin are mediated by the release of endogenous GH and insulin-like growth factor-1. Brzozowski *et al.*[18] reported that these effects involved vagal nerve integrity, partially depending upon afferent nerves and hyperemia mediated by sensory neuropeptides such as calcitonin gene-related peptide released from these nerves.

The most remarkable gastroprotective effects of ghrelin are supposed to be derived from its antioxidant effects. Eter *et al.*[19] reported that peripheral administration of ghrelin attenuated I/R-induced gastric mucosal injury by reducing ulceration, tissue congestion, cellular infiltration and vascular permeability in rats. Serum level of LDH and tissue content of tumor necrosis factor α (TNFα) were markedly reduced by the ghrelin administration. In their study, a decrement of thiobarbituric acid reactive substance (TBARS) and an increment in glutathione were observed, which suggested that ghrelin has an antioxidant activity. *In vitro* studies, using human polymorphonuclear cells incubated with ghrelin, showed that ghrelin inhibited ROS generation as measured by chemiluminecence.[19] Iseri *et al.*[21] reported that although alendronate induces oxidative gastric damage by a local irritant effect, ghrelin ameliorates this damage by its possible antioxidant and anti-inflammatory powers.

Ghrelin Secretion and Gastric Mucosal Injury

The most common causes of gastric mucosal injury and peptic ulceration are *Helicobacter pylori* (*H. pylori*) infection and the consumption of non-steroidal anti-inflammatory drugs (NSAIDs). *H. pylori* induces a strong inflammatory response, generating large amounts of ROS during the process of colonizing the host.[20,22–29] In the pathogenesis of NSAID-induced gastric mucosal injury, oxygen radicals also play an important role.[30]

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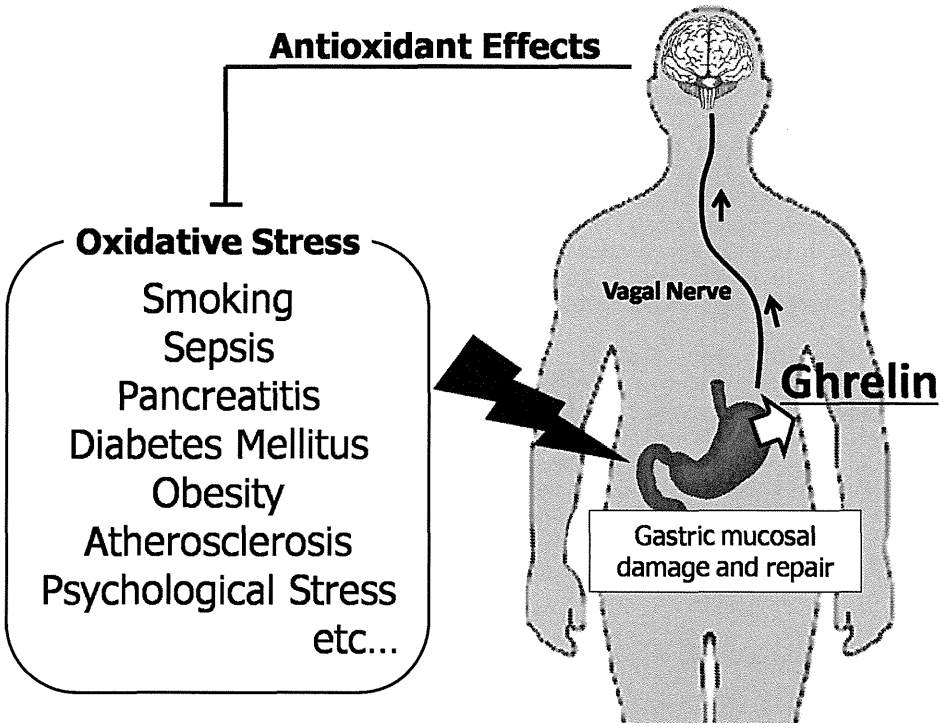

Fig. 1. Ghrelin has antioxidant effects on systemic oxidative stress. Many kinds of systemic oxidative stress could lead to gastric mucosal injury. Ghrelin would be released when the A-like cells were damaged or repaired. Secreted ghrelin send a signal to the brain through the vagal nerve, and enhance the antioxidant reaction in the body.

Although ghrelin secretion would be required to protect gastric mucosa against ROS-induced injury, the number of the gastric A-like cells is decreased simultaneously by gastric mucosal injury.[31,32] Therefore, the status of ghrelin secretion in gastric mucosal injury is complicated. The plasma total and active ghrelin levels are known to be increased by the formation of duodenal ulcers, which induced by administration of cysteamine, a somatostatin inhibitor, in rats.[33] In a human study, enhanced plasma ghrelin levels were observed in patients, not only with duodenal ulcers, but also with gastric ulcers.[34] According to the report by Isomoto *et al.*,[35] amomg plasma ghrelin levels of the patients with chronic gastritis, gastric ulcer, duodenal ulcer, acute gastritis, and normal mucosa, the levels of acute gastritis group were the highest, and then that of chronic gastritis group were the lowest. Within the *H. pylori*-positive population, the plasma ghrelin levels of duodenal ulcer group were higher than gastric ulcer group or chronic gastritis group.[35] The plasma total and active ghrelin levels correlated with the serum pepsinogen I levels, as well as the serum pepsinogen I/II ratio, and decreased with increasing extent of gastric mucosal atrophy.[36,37] These results suggest that the plasma ghrelin levels increase in response to the severe gastric mucosal oxidative stress. However, in the condition of *H. pylori*-induced gastric mucosal severe atrophy, the number of A like cells as well as the plasma ghrelin concentration shifted to lower levels with the reduction of other component cells in gastric fundic gland due to inflammatory cell infiltrations.

Gastric Motility Dysfunction and Ghrelin

Oxidative stress induces not only gastric mucosal injury, but also gastric motility dysfunction, such as diabetic gastroparesis. Gastroparesis are thought to be caused by ROS-induced damage of the networks of the interstitial cells of Cajal.[38] The authors reported that the plasma ghrelin levels and gastric preproghrelin mRNA expression levels are increased in rats with streptozotocin-induced diabetes, that is known for hyperphagia.[39] In a human study, however, the fasting plasma ghrelin level was significantly lower in diabetes mellitus with diabetic gastroparesis than in healthy controls.[40] The change in the plasma ghrelin levels with sham feeding in diabetic gastroparesis patients and postsurgical gastroparesis patients were significantly lower than in normal subjects, although the plasma ghrelin levels increased in idiopathic gastroparesis.[41] Impaired regulation of the plasma ghrelin levels in diabetic gastroparesis would be caused by vagal nerve dysfunction.[41]

Ghrelin administration accelerated gastric emptying of a meal in humans even in the presence of a neural dysregulation by diabetes or surgical vagotomy.[42] Also in idiopathic gastroparesis, administration of ghrelin enhances gastric emptying and improves meal-related symptoms.[43] Therefore, analogues of ghrelin are expected to represent a new class of prokinetic agents.[44] TZP-101, the synthetic, selective ghrelin agonist, is now tested in clinical trials. This new agent was well-tolerated in diabetes patients with moderate-to-severe chronic gastroparesis and showed statistically significant improvements in gastric emptying.[45,46]

Systemic Oxidative Stress and Ghrelin

Systemic oxidative stress is induced by various reasons. In diabetes mellitus, NADPH oxidases, endothelial NO synthase uncoupling, and protein kinase C signaling plays an important roles for mediating increased vascular superoxide production and endothelial dysfunction.[47] Smoking stimulates pulmonary alveolar macrophages and increased superoxide production.[48] During sepsis, multiple intracellular sites, such as mitochondrial, NADPH oxidase, and Rac1 pathways, are responsible to the superoxide production.[49]

Several studies suggested that systemic oxidative stress enhance the plasma ghrelin levels. The plasma ghrelin levels were correlated with vascular super oxide. production and NADPH oxidase

activity in patients with atherosclerosis.[50] Plasma ghrelin was elevated in cachectic patients with chronic heart failure, associated with increases in GH and TNFα.[51] Smoking acutely increased the plasma ghrelin levels.[52] On the other hand, in the early stage of sepsis, ghrelin levels decreased, although the activity of its receptor was markedly elevated in rats.[53] In patients with acute pancreatitis, the plasma ghrelin levels increased after patients' recovery, as compared with the levels before therapy.[54] Decreased ghrelin levels in the early phase of sepsis or pancreatitis would be caused by the damage of gastric A like cells. With repairment of the A like cells, the plasma ghrelin levels would recover after sepsis or pancreatitis.

Taken together, it is considered that gastric mucosa would play an important role for sensing a systemic oxidative stress (Fig. 1). Exposure to oxidative stress could lead to gastric mucosal injury, and ghrelin would be released when the A like cells were damaged or repaired. Secreted ghrelin would have an anti-inflammatory action on the oxidative injury of the several organs, such as increasing cardiac output,[55] vasorelaxation,[56] attenuation of acute pancreatic damage,[57] and attenuation of acute lung injury,[58] as well as rapid repairment of gastric epithelial cells. Ghrelin would be also secreted from the stomach as an anti-inflammatory player for the systemic oxidative injury.

Conclusions

Ghrelin has the possible antioxidant and anti-inflammatory effects. Selective ghrelin agonist would be expected as a new agent to treat not only gastrointestinal motility dysfunction, but also gastric mucosal injury, cardiovascular disease, and various systemic diseases induced by oxidative stress. The stomach would be an important organ as a sensor of systemic oxidative stress.

Abbreviations

ROS	reactive oxygen species
I/R	ischemia-reperfusion
GH	growth hormone
TBARS	thiobarbituric acid reactive substance
NSAIDs	non-steroidal anti-inflammatory drugs

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GASTROENTEROLOGY

Can *Helicobacter pylori*-associated dyspepsia be categorized as functional dyspepsia?

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Key words
acid, Asia, eradication, functional dyspepsia, ghrelin, *Helicobacter pylori*, meta-analysis, microRNA.

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Abstract

Evidence for an association between *H. pylori* and functional dyspepsia (FD) is uncertain. In the present review, we focused the special relevance of *H. pylori* infection to the development dyspepsia from the aspects of pathogensis, clinical efficacy of eradication of *H. pylori* in the West and in the East. Although clinical trials conducted to evaluate the efficacy of *H. pylori* eradication treatment for FD, including non-ulcer dyspepsia (NUD), have yielded conflicting results, it is quite clear that *H. pylori* eradication treatment is effective at least in a subset of FD patients. In contrast to the previous results obtained in studies of Western populations, the result of a double-blind, randomized, placebo-controlled trial conducted in a Singapore suggests that patients with FD could benefit from *H. pylori* eradication therapy, with as much as a 13-fold increase in the chance of symptom resolution. Especially in Asia, *H. pylori* should not be overlooked when considering the pathophysiology of FD. *H. pylori*-associated dyspepsia might be dealt as a different disease entity from FD.

Introduction

Functional dyspepsia (FD) refers to a broad range of chronic upper abdominal symptoms associated with food intake (pain or discomfort centered in the upper abdomen, early satiety, fullness, bloating sensation in the upper abdomen, nausea, etc),[1] and is a condition that is frequently encountered in clinical practice. It has been reported to occur at a prevalence rate of 15% in adults, and about 25% of those with the condition are estimated to be receiving some form of treatment.[2]

Since the etiology of dyspepsia is uncertain, it is little surprise that *H. pylori* (*Helicobacter pylori*) has been implicated in the pathogenesis of dyspepsia. Large population-based studies have shown that the bacterium is found more frequently in the gastric mucosa of dyspeptic patients than in that of controls.

Evidence for an association between *H. pylori* and FD is uncertain. Trials conducted to evaluate the efficacy of *H. pylori* eradication treatment for FD, including non-ulcer dyspepsia (NUD), have yielded conflicting results, however, it is quite clear that *H. pylori* eradication treatment is effective at least in a subset of patients with FD or NUD.[3,4]

Pathogenesis

There is some evidence to suggest that *H. pylori*-associated dyspepsia is caused by the effect of *H. pylori* infection on acid secretion. *H. pylori* infection is associated with increased fasting and post-prandial serum gastrin levels and decreased gastric mucosal concentrations of somatostatin, abnormalities which are corrected following *H. pylori* eradication.[5] Gastrin-releasing peptide (GRP) is a neuropeptide that induces responses mimicking the physiological responses of the stomach to a meal. After intravenous infusion of GRP, patients with peptic ulcers and *H. pylori* infection show a six-fold increase of acid secretion, patients with NUD and *H. pylori* infection show a four-fold increase of acid secretion, whereas asymptomatic *H. pylori*-positive individuals show only a $2^1/_2$ fold increase in stimulated acid secretion, as compared to asymptomatic controls without *H. pylori* infection.[6] This would suggest that NUD may represent a component of the spectrum of *H. pylori*-induced gastric disease. The spectrum ranges from an asymptomatic carrier state, through an ulcer-free dyspeptic period, finally to the development of peptic ulcer.

Ghrelin, a novel growth hormone-releasing peptide, has been reported to accelerate food intake and gastrointestinal motility. We investigated the plasma ghrelin levels in 47 patients with FD and 17 healthy controls. The plasma ghrelin levels were significantly higher in the FD patients, especially in those with dysmotility-like FD, based on the Rome II classification, as compared with those in the controls, suggesting that this parameter could become a useful novel supportive marker for the diagnosis of FD.[7] As the patient cohort with dysmotility-like FD is almost compatible to that with postprandial distress syndrome (PDS) described by the more recent Rome III classification, plasma ghrelin levels (total and active) are thought to be increased in subjects with PDS.

We recently investigated the role of microRNAs (miRNAs) in gastric motility disorders associated with *H. pylori* infection. The gastric motility was compared between mice with long-term *H. pylori* infection and uninfected mice. Gastric emptying was significantly accelerated in the mice with chronic *H. pylori* infection as well as those with infection by one of the other *Helicobacter* species, *H. felis*. Histologic examination showed that the muscular layer of the stomach was significantly thickened, with hyperplasia of the myocytes, in *H. pylori*-infected mice. The miRNA expression profile revealed that the muscle-specific miRNAs, miR-1, miR-133a and miR-133b were significantly downregulated in the stomachs of mice with long-term *H. pylori* infection. However, the expressions of histone deacetylase 4 (HDAC4) and serum response factor (SRF), which are reported as target genes of miR-1 and miR-133, and are known to enhance muscular hyperproliferation, were increased. Downregulation of miR-1 and miR-133 and increased cell proliferation were also observed in C2C12 mouse myoblast cells co-cultured with *H. pylori*. Chronic *H. pylori* infection is associated with downregulated expressions of muscle-specific miRNAs and upregulated expression of HDAC4 and SRF. These might cause hyperplasia of the muscular layer of the stomach and dysfunction of gastric emptying, especially accelerated gastric emptying, possibly through disturbed gastric accomodation. These findings provide a novel insight into the molecular pathogenesis of gastric motility disorders associated with *H. pylori* infection, and might show an organic aspect of *H. pylori*–associated dyspepsia.[8]

Further work has suggested that mast cells are found in patients with *H. pylori*- negative dyspepsia but not in *H. pylori*- positive patients, suggesting a different mechanism underlying the development of symptoms in patients with *H. pylori*-negative dyspepsia.[9]

H. pylori eradication

Many studies and several meta-analyses have attempted to establish a relationship between *H. pylori* infection and the development of FD (Table 1).

In 1998, McColl *et al.* performed a randomized, placebo-controlled trial comparing the efficacy of 2 weeks' treatment with omeprazole + antibiotics (160 patients) and omeprazole alone (158 patients) against the symptoms of dyspepsia in patients with *H. pylori* infection but no endoscopic evidence of ulcer disease. They reported that one year later, dyspepsia had resolved in 33 of the 154 patients (21%) given omeprazole plus antibiotics, as compared with 11 of the 154 (7%) patients given omeprazole alone (95% CI for the difference, 7 to 22%; $P < 0.001$), and that among the patients given omeprazole plus antibiotics, the symptoms resolved in 26 of the 98 patients (27%) who had had symptoms for five years or less, as compared with 7 of the 56 patients (12%) who had had symptoms for more than five years ($P = 0.03$). Their results clearly suggest that in patients with *H. pylori* infection and FD, *H. pylori* eradication treatment is more likely to resolve the symptoms than treatment with omeprazole alone.[10] On the other hand, Blum *et al.* conducted a double-blind, multicenter trial of patients with *H. pylori* infection and dyspeptic symptoms and showed that treatment was successful in 27.4% of the patients in the eradication treatment group and 20.7% in the group treated with omeprazole alone ($P = 0.17$) and concluded that in patients with FD, eradication of *H. pylori* is not likely to relieve symptoms.[11]

The different conclusions reached by two high-quality meta-analyses may be likely explained by which trials were included and which were excluded in each review.[12,13] Then, Talley *et al.*[14] randomly assigned 170 *H. pylori*-infected patients with non-ulcer dyspepsia (NUD) to receive twice-daily treatment with 20 mg of omeprazole, 1000 mg of amoxicillin, and 500 mg of clarithromycin for 14 days, and 167 such patients to receive identical-appearing placebos; all patients were then followed through regular visits for 12 months. They showed that at 12 months, there was no significant difference in the rate of successful treatment between the two groups (46% in the active-treatment group and 50% in the placebo group; relative likelihood of success with active treatment, 0.93; 95 percent confidence interval, 0.73 to 1.18; $P = 0.56$), and also found no evidence to suggest that eradication of *H. pylori* infection in patients with NUD would lead to relief of symptoms. Moayyedi *et al.*[15] also investigated the possibility of lowering the prevalence of dyspepsia in the community and improving the quality of life of the subjects by *H. pylori* eradication in a double-blind randomized controlled trial, and reported that community screening and treatment for *H. pylori* produced

Table 1 RCT for the effect of *H. pylori* eradication therapy on the treatment of functional dyspepsia (non-ulcer dyspepsia)

Authors	McColl *et al.*	Blum *et al.*	Talley *et al.*	Moayyedi *et al.*	Gwee *et al.*	Dhali *et al.*
Patient number (eradicated/non-eradicated)	154/154	164/164	150/143	880/871	41/41	32/30
Place	UK	Europe, Canada, South Africa	USA	UK	Singapore	India
Single/multi center	single center	multi-center	multi-center	field study	single center	single center
Eradication protocol	OAM	OAC	OAC	OCT	OCT	BTM
Control	omeprazole 40 mg/day	omeprazole 40 mg/day	placebos	placebos	placebos	sucralphate 4 g/day
Eradication period	14 days	7 days	14 days	7 days	7 days	14 days
Observation period	1 year	1 year	1 year	2 years	1 year	3 months
Effective rates (%) (eradicated/non-eradicated)	21/7	27.4/20.7	46/50	72/67	24/7	81/33
Efficacy	effective			effective	effective	effective

BTM, bismuth, tetracycline, metronidazole; OAC, omeprazole, amoxicillin, clarithromycin; OAM, omprazole, amoxicillin, metronidazole; OCT, omeprazole, clarithromycin, tinidazole.

only a 5% reduction in the prevalence of dyspepsia, with no impact on the QOL.

A recently published meta-analysis suggests that *H. pylori* eradication may have a small but statistically significant benefit in the treatment of FD at 12 months (relative risk of remaining dyspeptic after *H. pylori* eradication therapy = 0.91; 95% *CI* = 0.87–0.99). While statistically significant, the clinical significance of this finding is not so clear, because the effect is small, that is,15 *H. pylori-positive* dyspeptic patients will need to be treated to achieve just one case of relief from dyspepsia.[16]

H. pylori eradication in Asia

Several randomized controlled trials involving populations in the West have no statistically significant advantage of *H. pylori* eradiation therapy over placebo in dyspepsia patients. However, none of these studies involved Asian populations which show high infection rates.

In Singapore, Gwee *et al.* conducted a double-blind, randomized, placebo-controlled trial of *H. pylori* eradication therapy for FD in a Singapore-based Asian population. Forty-one patients received active treatment consisting of a 1-week course of omeprazole 20 mg once daily, clarithromycin 250 mg twice daily and tinidazole 500 mg twice daily, while another 41 patients received matching placebo tablets. On ITT analyses, symptom resolution was observed in 24% of the patients on active treatment and 7% of those on placebo; the difference in the proportion of patients showing symptom resolution between the two groups was statistically significant ($P = 0.02$, 95% confidence interval: 1.1–17.7). Among patients with *H. pylori* successful eradication, the symptom resolution rate was 39% (10/ 26 patients), whereas among patients in the placebo group who had persistent *H. pylori* infection, it was only 3% (1/35 patients).[17] In contrast to the results obtained in studies of Western populations, the results suggest that patients with FD in Asia could benefit from *H. pylori* eradication therapy, with as much as a 13-fold increase in the chance of symptom resolution following *H. pylori* eradication.

In India, Dhali *et al.*[18] performed a randomized study for evaluating the efficacy of anti-*H. pylori* treatment versus sucralphate in 62 patients with *H. pylori*-positive NUD. According to their report,[18] in patients of NUD and *H. pylori* infection, triple therapy eradicated *H. pylori* in 88% of the patients and was superior to sucralphate in producing symptom relief (81 *vs* 33%, $P = 0.0003$).

We have previously reported that successful *H. pylori* eradication improved the QOL of patients with FD, in particular *H. pylori*-positive patients with ulcer-like FD or dysmotility-like FD, in Japan.[19] The Gastrointestinal Symptom Rating Scale (GSRS) questionnaire was administered to the patient just before the start of the eradication therapy and at 3 months after the therapy, just before the UBT was performed. In successfully eradicated patients, significant decrease of the total GSRS and abdominal pain score was observed. In particular, significant decreases of the abdominal pain score and indigestion score were observed after successful *H. pylori* eradication in patients with ulcer-like FD or dysmotility-like FD.

Most studies on *H. pylori* in relation to dyspepsia have been performed on Western populations, and the results cannot be directly extrapolated to Asian populations, who differ from the western populations in many respects, including points about the most prevalent strains, average level of acid production, as well as the average severity and pattern of gastritis. *H. pylori* should not be overlooked when considering the pathophysiology of FD, especially in Asia.

Since FD is a highly heterogeneous disorder, traditional pathophysiological paradigms are still inadequate to explain the variations in the symptoms observed. Actually, current symptom classifications also largely failed to identify responders to specific therapies.

On the other hand, *H. pylori* infection evokes a significant level of inflammatory changes, not only in the gastric mucosa, but also in the gastric muscular layer as well as in the duodenum. The diagnosis of *H. pylori* infection seems rather homogeneous and it is not simple to categorize *H. pylori*-associated dyspepsia as an inorganic disease. Then, there might be a reason to consider *H. pylori*-associated dyspepsia as an organic disease and to deal with it as a different disease entity from FD. A new classification based on the mechanisms and specific symptoms needs to be considered to further the diagnostic and therapeutic advances in this field.

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Review

What Is the Difference Between *Helicobacter pylori*-Associated Dyspepsia and Functional Dyspepsia?

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Advances in basic and clinical research have revealed that *Helicobacter pylori* (*H. pylori*) infection plays an important role in the development of gastroduodenal dysmotility and hypersensitivity, as also in dyspepsia symptoms. In addition, recent studies have proposed an inflammation-immunological model for the pathogenesis of functional dyspepsia. Since *H. pylori* is the major microbe that provokes a gastroduodenal inflammatory response, it should not be overlooked when considering the pathophysiology of dyspepsia symptoms. In fact, population-based studies have demonstrated that *H. pylori* is detected more frequently in dyspepsia patients. However, although many clinical studies tried to reveal the association of *H. pylori* infection with gastric motility dysfunction or hypersensitivity, the results have been conflicting. On the other hand, many etiological features were revealed for the development of *H. pylori*-associated dyspepsia, such as abnormal ghrelin or leptic secretion, altered expression of muscle-specific microRNAs, and duodenal inflammatory cell infiltration. In addition, therapeutic strategy for *H. pylori*-associated dyspepsia would be different from *H. pylori*-negative functional dyspepsia. This review focuses the issue of whether *H. pylori*-associated dyspepsia should be considered as a different disease entity from functional dyspepsia.
(J Neurogastroenterol Motil 2011;17:124-130)

Key Words
Duodenum; Ghrelin; *Helicobacter pylori*; MicroRNAs

Introduction

Functional dyspepsia (FD) is a syndrome characterized by chronic and recurrent gastroduodenal symptoms in the absence of any organic or metabolic disease that is likely to explain the symptoms.[1,2] FD is considered to be important to public health, because it is remarkably common, can be disabling, and can pose a major social and economic burden.[3] Since FD is a highly heterogeneous disorder, numerous pathophysiological mechanisms, such as gastroduodenal motor dysfunction, visceral hypersensitivity, central nervous system dysfunction, *Helicobacter pylori* (*H. pylori*) infection and psychosocial factors have been suggested to play a role in the development of FD.

Although numerous epidemiological trials have suggested a higher prevalence of *H. pylori* infection in FD patients, the results have been conflicting.[4] Results of a meta-analysis showed that the prevalence of *H. pylori* infection was greater in patients with dys-

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pepsia than in controls, with an odds ratio of 2.3 (95% CI, 1.9-2.7).[5] Although this result seems to support the role of *H. pylori* infection in the pathogenesis of dyspepsia, it appears that some of the studies that were included in the analysis were biased by the selection of controls not properly matched for age, socioeconomic status and ethnic background.[4]

However, recent studies have revealed a subset of patients who developed FD after an episode of gastrointestinal infection. These studies, proposing the concept of post-infectious FD, suggest that an inflammation-immunological circuit also plays an important role in the development of FD.[6] It is generally well-recognized that the major cause of gastroduodenal inflammation is *H. pylori* infection.[7] Since *H. pylori* induces activation of a complex and fascinating cytokine and chemokine network in the gastric mucosa,[8] it is of little surprise that *H. pylori* infection has been implicated in the pathogenesis of dyspepsia.

For this reason, one of the major research interest is the difference between *H. pylori*-associated dyspepsia and other functional dyspepsia.[9] In this review article, FD in patients with a present or even past history of *H. pylori* infection is defined as a different disease entity (*H. pylori*-associated dyspepsia [HpD]) from FD, especially by focusing on the etiological insight of HpD, and then discusses the therapeutic strategy of HpD.

Influence of *Helicobacter pylori* Infection on Dyspepsia Symptoms and the Gastric Functions

A lot of clinical evidences have been published to investigate whether *H. pylori* infection is involved in gastric motility disorders and visceral hypersensitivity. However, all of these studies were small-scale studies, and the results were conflicting. Few studies have shown the association between gastric visceral hypersensitivity and *H. pylori* infection. Thumshirn et al[10] compared gastric motor and sensory functions in 17 patients with FD and 16 asymptomatic controls, and reported that *H. pylori* infection did not appear to influence gastric accommodation, but was associated with hypersensitivity in FD patients. On the other hand, some researchers were able to show the association between gastric motility dysfunction and *H. pylori* infection. Mearin et al[11] investigated the symptomatic pattern in 27 *H. pylori*-positive and 23 *H. pylori*- negative patients with FD, and showed that FD patients with *H. pylori* infection presented no distinctive symptoms in comparison with their *H. pylori*-negative counterparts, and that *H. pylori* infection was associated with diminished postprandial antral motility, but did not increase the perception of gastric distension. Tucci et al[12] evaluated the *H. pylori* infection status, histological features of the gastric mucosa, and the gastric motor and secretory functions in 45 consecutive patients with FD. *H. pylori* infection was found in 60% of FD patients, as compared with 33% of the 15 healthy controls. No difference was detected in the basal or stimulated gastric acid secretion between the FD patients and healthy controls. Gastric emptying was significantly delayed in FD patients as compared with that in healthy controls after adjustments for age and sex. Delayed gastric emptying was associated with a low frequency of *H. pylori* infection, female gender and young age. Epigastric pain or burning and postprandial fullness were more severe in patients with *H. pylori* infection and in those with delayed gastric emptying, respectively. Saslow et al[13] compared 8 *H. pylori*- positive and 8 *H. pylori*-negative asymptomatic subjects, and showed that *H. pylori* infection reduced accommodation, but had no effect on the overall sensation or motor functions of the stomach. However, some studies showed that *H. pylori* infection did not affect gastric motility or hypersensitivity. Leontiadis et al[14] evaluated 23 FD patients and 17 controls, and showed that although gastric emptying was delayed in FD patients, the gastric emptying rate was not associated with the *H. pylori* infection status, and was also not affected by eradication of the infection. Chang et al[15] compared 22 *H. pylori*- negative patients and 38 *H. pylori*- positive patients with FD, and showed that the *H. pylori* infection status appeared to have no influence on the incidence of delayed gastric emptying of digestible and indigestible solids.

Although the results of several clinical studies suggest that *H. pylori* infection may play a role in the development of FD, the precise pathogenesis of HpD could not be elucidated. Since gastric dysmotility and visceral hypersensitivity are induced by a number of confounding factors, such as diet, smoking and psychosocial stress, the association of *H. pylori* infection with gastric sensation or motor dysfunction might be difficult to be revealed only by clinical studies. A large-scale clinical study controlled for all of these factors would be difficult to design. Thus, novel biological markers for HpD other than gastric dysmotility and hypersensitivity must be identified. On next section, therefore, the possible pathophysiology of HpD will be reviewed.

Pathophysiological Link Between *Helicobacter pylori* Infection and Dyspepsia

Traditionally, gastric acid hypersecretion induced by *H. pylori*

infection of the gastric antral mucosa has been considered to play a role in the development of dyspepsia. About 10%-15% of patients with *H. pylori* infection show antral-predominant gastritis, which results in gastric acid hypersecretion.[16] In these patients, *H. pylori* induced a decrease in somatostatin secretion in the antral gland area, leading to an increase in the release of gastrin and subsequently to a rise in acid secretion.[17] This mechanism is also considered to underlie the development of duodenal ulcer. These phenomena are reversible, since normal feedback control of gastrin secretion is restored after *H. pylori* eradication.[17,18]

However, a few studies investigating the association between the severity of histological gastritis and that of dyspepsia symptoms yielded different results. Turkkan et al[19] reported that dyspepsia symptom scores were higher in patients with mild or moderate chronic inflammation of the corpus and antrum than in those with severe chronic inflammation, although the difference did not reach statistical significance. In studies conducted by Joshi et al[20] and Pereira-Lima et al,[21] no relationship was found between the severity of histological gastritis and the severity of the dyspeptic symptoms. Czinn et al[22] found a relationship between epigastric pain and the severity of inflammation. Similarly, van der Schaar et al[23] also found an indirect relationship between the severity of symptoms and the severity of inflammation of the corpus. From these results, we could not reach any definitive conclusion about the association of severity of gastritis or amount of gastric acid secretion with severity of the dyspepsia symptoms.

Ghrelin, which is produced and secreted by the A-like cells of the oxyntic glands of the stomach, has a well-established role in increasing appetite and food intake and in stimulating gastric emptying and acid secretion.[24-28] These functions are mediated, at least in part, via vagal nerve pathways.[29,30] In gastroduodenal mucosal injury, the levels of plasma ghrelin increased in response to the physiological demand for the purpose of gastroduodenal cytoprotection.[31,32] However, in the presence of *H. pylori*-induced severe gastric mucosal atrophy, the plasma ghrelin concentrations shifted to lower levels.[33-36] Taken together, *H. pylori* infection may induce gastric motor dysfunction and reduce appetite with suppressed ghrelin secretion. Therefore, this peptide may play a role in the onset of FD, especially HpD. In fact, alterations of the plasma ghrelin levels have been reported in FD patients, which frequently correlated with the FD symptom score.[37-39] Some studies showed that plasma ghrelin levels were significantly lower in patients with dysmotility-like FD.[28,37] Concerning the active ghrelin levels, they were also decreased in patients with postprandial fullness and/or early satiation,[40] whereas similar between dysmotility-like FD patients and healthy controls.[37] Moreover, recent study showed that repeated ghrelin administrations had stimulatory effects on food intake in FD patients.[41] However, the opposite results, such as enhanced ghrelin levels in FD patients, were also reported.[38,42] Leptin is also produced in the stomach, and activates vagal nerve ternimals, reduces appetite and increases mucin secretion.[43] Leptin may also play a role in the onset of FD, since patients with dysmotility-like dyspepsia have been reported to show higher serum concentrations of leptin.[44] On the other hand, serum leptin levels and expression of leptin mRNA in the gastric mucosa was enhanced in *H. pylori*-positive patients,[44,45] suggesting that *H. pylori* infection may reduce appatite with enhanced leptin secretion. The circulatory levels of ghrelin and leptin in HpD patients have not yet been investigated, warranting future research.

We recently investigated the role of microRNAs (miRNAs) in gastric motility disorders associated with *H. pylori* infection,[46] and the results provided a novel insight into the molecular pathogenesis of HpD. Histologic examination showed prominent thickening of the muscular layer of the gastric corpus in *H. pylori*-infected mice. In addition, gastric emptying was significantly accelerated in *H. pylori*-infected mice. The miRNA expression profile revealed that the muscle-specific miRNAs, *miR-1, miR-133a* and *miR-133b*, were downregulated in the stomach of *H. pylori*- infected mice. The expression levels of histone deacetylase 4 and serum response factor, which are target genes of *miR-1* and *miR-133* known to enhance muscular hyperproliferation, were increased. Taken together, chronic *H. pylori* infection downregulates the expressions of muscle-specific miRNAs and upregulates the expression of histone deacetylase 4 and serum response factor, which might cause hyperplasia of the muscular layer of the stomach and deregulation of gastric emptying in mice. Further human studies will be necessary to validate the association between aberrant expression of muscle-specific miRNAs in the muscular layer of the stomach and HpD.

Duodenum - A Crossroad Between *Helicobacter pylori* and Dyspepsia

Recent studies have emerged implicating abnormal motor and autonomic responses in the duodenum perhaps triggering functional responses, including pain and abnormal gastric emptying. Increased duodenal acid exposure has been reported in patients with dyspepsia symptoms. At the level of the duodenum, abnormalities may exist in the stimulus intensity, mucosal

mRNA expression, biosynthesis, release or inactivation of the mucosal mediators, or in the receptor expression on the afferent nerve endings.[47]

Furthermore, Talley et al[48] proposed that changes in the duodenal eosinophil count might be an underlying feature of FD. They also showed that eosinophils were significantly increased in both the bulb and second portion of the duodenum in FD, whereas increase of the mast cells in the second portion of the duodenum was noted in irritable bowel syndrome (IBS).[49,50] A link between eosinophils (and other inflammatory cells) and FD would have therapeutic implications. Eosinophils are critically dependent on the cytokine IL-5 for their maturation in the bone marrow, which also influences eosinophil migration and survival. Kindt et al[51] reported that stimulated lymphocyte expression of IL-5 and IL-13 was enhanced, whereas stimulated monocytic IL-12 and lymphocytic IL-10 expression were reduced in both FD and IBS. Based on these findings, anti-inflammatory agents, possibly including novel biologics such as anti-IL-5 humanized antibodies, could be explored as a possible therapeutic candidates for FD.

Active duodenitis has been reported to be more common in patients with *H. pylori* infection.[52] Genta et al[52] reported that *H. pylori* was detected in the gastric metaplastic epithelium of 67.6% of patients with active inflammation of the duodenum. On the other hand, *H. pylori* infection is well-known to cause eosinophil infiltration of the gastric mucosa.[53] Taken together, *H. pylori* might be one of the causes of duodenal eosinophilia, as well as of the onset of dyspepsia symptoms.

In addition, Gargala et al[54] reported that the number of intraepithelial lymphocytes in the duodenal mucosa was significantly greater in *H. pylori*-positive FD patients than in healthy controls, but not different between *H. pylori*-negative FD patients and healthy controls. The expressions of CD95/Fas and HLA-DR-expressing $CD3^+$ lymphocytes were lower in *H. pylori*-negative FD patients than in healthy controls. These findings suggest that the phenotypic characteristics of intraepithelial lymphocytes may be different between HpD and *H. pylori*-negative FD.

Treatment for *Helicobacter pylori*-Associated Dyspepsia

Although a number of clinical trials have assessed the efficacy of *H. pylori* eradication for the treatment of FD, the studies drew different conclusions. However, it is quite clear that *H. pylori* eradication treatment is effective in at least a subset of patients with FD.[7,55-58] According to a meta-analysis of randomized controlled trials to determine the effect of *H. pylori* eradication on dyspepsia symptoms, *H. pylori* eradication therapy appears to have a small but statistically significant effect in HpD.[59] Harvey et al[60] showed that *H. pylori* eradication gave cumulative long-term benefit, with a continued reduction in the development of dyspepsia severe enough to require a consultation with a general practitioner up to at least 7 years.

The efficacy for patients with HpD in Asia would be different from those in Western countries, since Asian population differs from the Western population in many respects, such as prevalent *H. pylori* strains, including cagA gene polymorphisms, levels of acid secretion in the stomach and the severity or pattern of gastritis.[58,61] In fact, Gwee et al[62] showed that the patients with FD in Asia would have a benefit from treatment for *H. pylori* infection with as much as a 13-fold increased chance of symptom resolution following its eradication in a double blind, randomized and placebo-controlled trial in Singapore-based Asian population.

There is no evidence of treatment for HpD patients after the successful eradication of *H. pylori*. At present, acid suppression is a frequently used first-line therapy for FD. A meta-analysis of randomized controlled trials of proton pump inhibitors (PPIs) for FD reported that this class of agents was superior to placebo.[63] However, much of this benefit may be explained by the presence of concomitant unrecognized gastroesophageal reflux disease (GERD). Xiao et al[64] showed that the prevalence of pathologic esophageal acid reflux without typical reflux symptoms (silent reflux) was 31.7% in FD patients. In addition, PPIs were effective in 83.1% of FD patients with silent reflux, and in 54.3% of those without silent reflux. On the other hand, inverse associations are observed between the presence of *H. pylori* infection and GERD, because of the reduction in gastric acid production by *H. pylori* colonization of the gastric mucosa.[65,66] This suggests that the efficacy of PPIs in HpD may be weaker than that in *H. pylori*-negative FD, which may show strong overlap with GERD.

On the other hand, a gastro-protective agent for chronic gastritis would be a therapeutic candidate for HpD. Rebamipide, a gastro-protective anti-ulcer drug, has been used for the improvement of dyspepsia symptoms in Japan, Korea, China and some other countries. Rebamipide is known to suppress gastric mucosal inflammation, which is thought to be related to its activity in the inhibition of superoxide anion production from neutrophils and scavenging hydroxyl radicals.[67,68] Rebamipide administration after *H. pylori* eradication could promote the restoration of atro

phic mucosa in Mongolian gerbils.[69] Chitapanarux et al[70] reported that rebamipide treatment improved symptom, endoscopic and histologic features of chronic gastritis in patients with dyspepsia symptoms refractory to PPIs. Talley et al[71] reported a double-blind, placebo-controlled and multicenter study of rebamipide for the treatment of FD patients with or without *H. pylori* infection. Although a significant improvement of individual symptoms at 8 weeks was not detected, the ratio of patients who requested usage of the study medication again was greater in the rebamipide groups compared with the placebo group in *H. pylori*-positive patients. During the planning of this study, it was originally projected that a sample size of 100 patients per treatment group would be sufficient to detect a difference in response rate of approximately 20% between the rebamipide treatment group and the placebo treatment group with 80% power at the 0.05 significance level. However, because of the slow patient recruitment and unexpected budget constraints, the trial had stopped prior to completion of enrollment. Based on the enrolled population of approximately 50 patients per arm in the *H. pylori*-negative study and 30 patients per arm in the *H. pylori*-positive study, the detectable differences would be 30% and 40%, respectively. The 30% superiority over the placebo would be non-realistic hurdle for any medication for FD. Miwa et al[72] also reported a double-blind, placebo-controlled and single-center study of rebamipide for the treatment of FD patients. Although the mean changes in overall symptoms after 4 weeks of treatment were not significantly different between the rebamipide and placebo treatment groups, the improvement in symptom score was significantly greater in the rebamipide group for bloating, belching and pain or discomfort that was relieved after a meal. Social restriction and pain intensity were also improved in the rebamipide group. The ratio of subjects with *H. pylori* infection were 54.1% in the rebamipide group and 42.4% in the placebo group. However, they did not perform subanalysis by *H. pylori* status as the number of subjects was rather small. As rebamipide has an anti-inflammatory effect, it might be effective for HpD, but not for FD patients without gastritis. However, there is not enough evidence for the efficacy of rebamipide for dyspepsia symptoms of HpD patients.

Therefore, the efficacy of all the existing medical treatment, including a gastro-protective agent, for FD should be re-evaluated for HpD and *H. pylori*-negative FD. Well-designed studies to investigate a suitable therapeutic strategy for HpD are needed.

Conclusions

Several mechanisms have been postulated for the development of HpD. Some of these mechanisms would be reversible, while others might not. Therefore, it would be reasonable that the *H. pylori* "test-and-treat" strategy is not effective in all HpD patients, but is effective in only a subset of HpD patients. *H. pylori* infection evokes significant inflammatory changes, not only in the gastric mucosa, but also in the gastric muscular layer as well as in the duodenum. However, most patients with *H. pylori* infection do not have any symptoms. We therefore need to conduct further investigation about the true relationship between dyspepsia symptoms and *H. pylori* infection to determine whether there might be identifiable risk factors for the onset of symptoms.

When the Rome III criteria were developed, the role of *H. pylori* infection in FD was controversial. Now, however, the pathophysiology underlying disturbances of gastroduodenal motor or sensory function and dyspepsia symptoms caused by *H. pylori* infection is gradually being elucidated. Therefore, when HpD is considered as an organic disease and as a different disease entity from FD, these conflicting results of previous studies might become more comprehensible. Further studies will be necessary to determine whether HpD should be separated from FD. In addition, the differences in the therapeutic strategies between HpD and *H. pylori*-negative FD are also necessary to be investigated in the future.

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Enhancement of Amoxicillin Resistance after Unsuccessful *Helicobacter pylori* Eradication▽

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A high rate of resistance (49.5 to 72.7%) to amoxicillin (AMX) was observed in *Helicobacter pylori* after two or three unsuccessful eradication attempts. Unsuccessful eradication regimens significantly increase resistance to not only clarithromycin (CLR) and metronidazole (MNZ) but also AMX.

Currently available eradication regimens for *Helicobacter pylori* are triple-drug combination regimens comprising a proton pump inhibitor (PPI) and two antibiotic drugs, and clarithromycin (CLR), metronidazole (MNZ), and amoxicillin (AMX) are commonly used antibiotics (12). Although *H. pylori* bacteria easily become resistant to CLR and MNZ, *H. pylori* has been thought to seldom become resistant to AMX (6). In the present study, the resistance rates after unsuccessful eradication attempts were examined.

A total of 343 patients (189 males and 154 females; mean age, 55.8 years) with *H. pylori* infection were enrolled between September 2004 and October 2010. *H. pylori* infection was defined by *H. pylori* culture positivity. Of the total, 22 patients had no history of antibacterial therapy for eradication, 211 patients had one treatment failure, 99 patients had two treatment failures, and 11 patients had three treatment failures (first-line treatment, triple therapy with CLR [800 mg/day], AMX [1,500 mg/day], and PPI for 7 days; second-line treatment, triple therapy with MNZ [500 mg/day], AMX [1,500 mg/day], and PPI for 7 days; third-line treatment, triple therapy with fluoroquinolone [levofloxacin, 400 mg/day; gatifloxacin, 400 mg/day; or sitafloxacin, 400 mg/day], AMX [2,000 mg/day], and PPI for 7 days) (8, 13). All patients underwent esophagogastroduodenoscopy and gastric biopsy for bacterial culture 6 to 12 months after the eradication failure at Keio University Hospital and National Tokyo Medical Center.

TABLE 1. Eradication failures and resistance rates

Agent	Prior treatment	% resistance (no. of resistant strains/no. tested)[a]			MIC of agent		
		With AMX MIC (μg/ml) of:		Other resistance			
		≥0.06	≥0.5		50%	90%	Range
AMX	None	13.6 (3/22)	0 (0/22)		<0.015	0.06	<0.015–0.12
	One failure	26.5 (56/211)	0.9 (2/211)		<0.015	0.12	<0.015–0.5
	Two failures	49.5 (49/99) ++ ###	6.1 (6/99) #		0.03	0.25	<0.015–4
	Three failures	72.7 (8/11) +++ ##	18.2 (2/11) ##		0.12	0.5	<0.015–4
CLR	No treatment			9.1 (2/22)	0.03	0.25	<0.015–8
	One failure			89.6 (189/211) +++	16	32	<0.015–64
	Two failures			88.8 (88/99) +++	16	32	<0.015–64
	Three failures			72.7 (8/11) +++	16	64	<0.015–64
MNZ	None			13.6 (3/22)	2	8	0.25–32
	One failure			4.7 (10/211)	1	2	0.5–32
	Two failures			72.7 (72/99) +++ ###	16	64	1–64
	Three failures			72.7 (8/11) ++ ###	16	32	4–32

[a] AMX resistance, MIC ≥ 0.06 μg/ml; AMX high-level resistance, MIC ≥ 0.5 μg/ml; CLR resistance, MIC ≥ 1μg/ml; MNZ resistance, MIC ≥ 8 μg/ml. ++, $P < 0.01$ versus results for nontreatment group; +++, $P < 0.001$ versus results for nontreatment group; #, $P < 0.05$ versus results for one-failure group; ##, $P < 0.01$ versus results for one-failure group; ###, $P < 0.001$ versus results for one-failure group.

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TABLE 2. Substitutions in penicillin binding protein 1 of *H. pylori* strains

Strain or group (n^a)	AMX MIC (μg/ml)	Substitution at penicillin binding protein 1 position:						
		374	406	414	535	593	599	601
KS0461	4				Asp		Gly	Gly
KS0478	0.5				Asp			
KS0487	0.5	Leu		Arg				
KS0439	0.25		Ala			Ala		
KS0476	0.25				Asp		Gly	
KS0470	0.25				Asp			
KS0444	0.12		Ala			Ala		
KS0464, KS0479	0.12				Asp			
KS0493	0.12						Pro	
KS0434	0.06		Ala					
KS0466, KS0491	0.06				Asp			
KS0503	0.06			Arg				
KS0502	0.06							
AMX-susceptible strains (15)	≤0.03	Val	Glu	Ser	Asn/Asp[b]	Thr	Ala	Val

[a] *n*, no. of strains.
[b] $535_{Asn \rightarrow Asp}$ was detected in the amoxicillin-susceptible strains KS0447, KS0452, and KS0467.

TABLE 3. Amoxicillin resistance rate and susceptibility to clarithromycin and metronidazole

Resistance	% amoxicillin resistance (no. of resistant strains/no. tested)[a]	MIC	
		50%	90%
CLR susceptible, MNZ susceptible	13.6 (6/45)	<0.015	0.06
CLR resistant, MNZ susceptible	32.2 (66/205) +	0.03	0.12
CLR susceptible, MNZ resistant	45.5 (5/11) +	0.03	0.5
CLR resistant, MNZ resistant	48.8 (40/82) # +++	0.03	0.25

[a] Amoxicillin resistance: MIC ≥ 0.06 μg/ml. +, $P < 0.05$ versus results for CLR-susceptible, MNZ-susceptible group; +++, $P < 0.001$ versus results for CLR-susceptible, MNZ-susceptible group; #, $P < 0.05$ versus results for CLR-resistant, MNZ-susceptible group.

Susceptibilities of *H. pylori* isolates to AMX, CLR, and MNZ were determined by the agar dilution method according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (1, 7). Isolates were considered resistant to MNZ if the MIC of the drug was ≥8 μg/ml and to CLR if the MIC was ≥1 μg/ml (9). For AMX, the interpretive standard (susceptible, ≤0.03 μg/ml) established by the Japanese Society of Chemotherapy was used (3). Isolates were defined as high-level resistant and resistant to AMX if the MIC was ≥0.5 μg/ml and ≥0.06 μg/ml, respectively (11), in this study. Differences between groups were compared by Fisher's exact test or the chi-squared test.

The rates of resistance to AMX in the groups with no history of eradication treatment, one treatment failure, two treatment failures, and three treatment failures were 13.6%, 26.5%, 49.5%, and 72.7%, respectively. The high-level rates of resistance to AMX in the group with no history of eradication treatment, one treatment failure, two treatment failures, and three treatment failures were 0%, 0.9%, 6.1%, and 18.2%, respectively (Table 1). The rates of resistance to AMX in the group with two treatment failures and that with three treatment failures were significantly higher than that in the group with no history of eradication treatment and that with one treatment failure. To the best of our knowledge, the present study is the first to report the increase in rates of resistance to AMX after unsuccessful *H. pylori* eradication.

The MIC_{90} of AMX showed 2-fold increases with every eradication failure. The MIC_{90} of CLR showed a 128-fold increase after triple therapy with CLR, AMX, and PPI, and the MIC_{90} of MNZ showed a 32-fold increase after triple therapy with MNZ, AMX, and PPI (Table 1). While the 23S rRNA point mutation is a main cause of CLR resistance (4) and the single mutation of *rdxA* or *frxA* is one of the main causes of MNZ resistance (5), multiple mutations in penicillin binding protein 1 (PBP1) would contribute to a greater increase in the level of AMX resistance (11) and then could result in a gradual increase in AMX resistance.

We amplified the bacterial DNA by PCR and sequenced the *PBP1* genes in 30 strains between September 2008 and April 2010 (forward, 5′-CACRAGCACCGGTAAGATTT-3′; reverse, 5′-GCGACAATAAGAGTGGCA-3′). The sequences obtained were compared with the published sequences of *H. pylori PBP1* (L26695; GenBank accession number AE000511). Table 2 shows the substitutions detected in AMX-resistant strains. Strains with high-level resistance to AMX had 1 to 3 substitutions, and low-level-resistant strains (MICs of 0.06 to 0.25 μg/ml) had 0 to 2 substitutions. The accumulation of *PBP1* mutations could result in a gradual increase in AMX resistance. The $Asn_{535} \rightarrow Asp$ substitution was also detected in not only AMX-resistant strains but also 3 of 15 (20%) AMX-susceptible strains.

The AMX resistance rates were 13.6% (6/45) in the strains susceptible to both CLR and MNZ, 32.2% (66/205) in the strains resistant to CLR but susceptible to MNZ, 45.5% (5/11) in the strains resistant to MNZ but susceptible to CLR, and 48.8% (40/82) in the strains resistant to both CLR and MNZ. The AMX resistance rate in the strains resistant to CLR or MNZ was significantly higher than that in the strains susceptible to both CLR and MNZ. The rate of resistance to AMX in the strains resistant to both CLR and MNZ was significantly higher than that in the strains susceptible to MNZ (Table 3). Efflux pump systems in bacteria, which can eject drugs and toxic compounds, including antibiotics, have a critical role in the development of multidrug resistance. We recently reported that the expression of the TolC efflux pump (*hefA*) was significantly increased under MNZ exposure (14). The efflux pump of *H. pylori* is also associated with the development of resistance to CLR, in addition to 23S rRNA point mutations (2). In addition to the known mutations in the gene coding for PBP, activated efflux systems may also play a role in *H. pylori* resistance to AMX.

In conclusion, contrary to our expectations, resistance to AMX in *H. pylori* was gradually induced after unsuccessful eradication attempts. The data are clearly consistent with the association of resistance rates and eradication failures. If AMX-resistant *H. pylori* strains were to spread further, serious problems would arise, resulting in increasing eradication failures (10). Our results suggest that clinicians should be aware of