Short Communications

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Characteristics of Successful Third-Line Eradication of *Helicobacter pylori* based on Antibiotic Susceptibility

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Abstract

Background: Regimens using vonoprazan (VPZ), amoxicillin (AMX), and sitafloxacin (STFX) have been reported to be effective for the third-line eradication of *Helicobacter pylori* and are widely used in Japan. However, the outcomes of these therapies have only been reported by a limited number of institutions. We aimed to investigate the backgrounds and treatment outcomes of patients who underwent successful third-line eradication of *H. pylori*. **Methods:** This was a single-center, retrospective, observational study included patients who underwent antibiotic susceptibility testing and third-line eradication of *H. pylori* between 2013 and 2020. We examined the success rates of the third-line regimens and compared the backgrounds and clinical characteristics of patients between the successful and failed eradication groups. **Results:** Fifty-three patients were enrolled. The overall success rate of third-line eradication was 79.2% (42/53 patients). The most commonly used regimens included proton pump inhibitors (PPI) or VPZ, AMX, and STFX (n=43). The success rate of the VPZ, AMX, and STFX regimens was 89.7% (26/29), whereas that of the PPI, AMX, and STFX regimens was 64.3% (9/14). In the comparison between the success and failure groups, the number of female patients (P=0.001) and AMX sensitivity (P=0.028) were significantly higher in the success group than in the failure group. VPZ use and STFX sensitivity were also more common in the successful group, although the differences were not significant. **Conclusion:** VPZ, AMX, and STFX regimens were the most common and effective regimens, with female and AMX-sensitive patients more likely to have successful third-line eradication.

Keywords: Antibiotic susceptibility- *Helicobacter pylori*- Third-line eradication

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Introduction

Helicobacter pylori infection is a leading cause of gastric cancer, and eradication therapy is recommended for positive cases [1]. For *H.pylori* eradication therapy, vonoprazan (VPZ) was first made available for use in Japan in 2015. Since then, it has become available in East Asia and the United States. European guidelines recommend fluoroquinolone antibiotics as rescue therapy in cases of second-line eradication failure [1]. Among the various options, sitafloxacin (STFX) has demonstrated notable efficacy and is extensively utilized in Japan [2-4]. However, reports on the outcomes of third-line *H. pylori* eradication remain scarce and are limited to a few institutions in the country. Moreover, there are limited data on antibiotic susceptibility tests to select a third-line eradication regimen [5, 6]. In this study, we assessed the selection of regimens for third-line eradication at our institution, evaluated their success rates, and identified the factors associated with successful outcomes. This analysis was based on the clinical characteristics and antimicrobial susceptibility test results.

Materials and Methods

Study design and population

This was a single-center, retrospective, observational study included 53 patients who underwent third-line eradication of *H. pylori* at our hospital from December 2013 to December 2020 and who underwent antibiotic susceptibility testing from gastric biopsy specimens.

Assessment of H. pylori infection, antibiotic susceptibility test, and cut-off points for antimicrobial resistance to H. pylori

The presence of *H. pylori* prior to third-line eradication therapy was confirmed by culture using two tissue samples from the greater curvature of the antrum and greater curvature of the middle part of the gastric body. The minimum inhibitory concentrations (MICs) of amoxicillin

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(AMX), clarithromycin (CAM), levofloxacin (LVFX), STFX, and metronidazole (MTX) against *H. pylori* isolates were determined using an agar dilution method. The cut-off points for antimicrobial resistance to *H. pylori* were defined as 0.06 μg/mL for AMX, 1.0 μg/mL for CAM, 0.5 μg/mL for LVFX, 0.12 μg/mL for STFX, and 8.0 μg/mL for MTX in accordance with the data in previous reports [7, 8].

Regimen of third-line eradication therapy and assessment of eradication success

Patients were administered one of the following gastric acid inhibitors: proton pump inhibitor (PPI) (esomeprazole at 20 mg twice daily [b.i.d.], rabeprazole at 20 mg [b.i.d.], omeprazole at 20 mg [b.i.d.], or lansoprazole at 30 mg [b.i.d.]) or VPZ at 20 mg [b.i.d.]. The physician selected two or three antibiotics: AMX (750 mg, b.i.d.), STFX (100 mg, b.i.d.), CAM (200 mg, b.i.d.), MTZ (250 mg, b.i.d.), and minocycline (MINO) (100 mg, b.i.d.). Eight-to-twelve weeks after the completion of the third-line eradication treatment, successful eradication was confirmed using a 13C urea breath test (UBT). The cutoff value for a negative UBT was <2.5‰ [9].

Evaluations

The type of regimen selected and the third-line eradication rates were evaluated. We compared the following patient characteristics between the successful and failed eradication groups in patients who were treated with regimens including AMX and STFX, the most frequently prescribed antibiotics: PPI or VPZ use, sex, age, history of smoking, presence or absence of AMX sensitivity, and presence or absence of STFX resistance.

Statistical analysis

Statistical analyses were performed using EZR (Easy R) version 1.61 (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Quantitative data were presented as medians and interquartile ranges, as appropriate. Fisher's exact test was used to analyze qualitative data, and the Mann–Whitney U test was used to analyze continuous data. Statistical significance was defined as a two-sided P-value <0.05

Results

Patient backgrounds

The characteristics of the 53 patients who underwent third-line eradication therapy, type of regimen selected, and eradication rates are shown in Table 1. The median age of the enrolled patients was 52 (interquartile range [IQR], 44–65 years) years. Eighteen were male, 35 were

Table 1. Patient Characteristics, Regimen Selected for Third-Line Eradication, and Eradication Rate

Patient characteristics		
Patient, No.	53	
Age, year (median [IQR])	52 (44–65)	
Sex, n (%)		
Male	18 (34.0%)	
Female	35 (66.0%)	
Smoking, n (%)		
+	14 (26.4%)	
–	20 (37.7%)	
unknown	19 (35.8%)	
Antibiotic susceptibility test, n (%)		
Not sensitive to AMX	9 (17.0%)	
Resistant to CAM	51(96.2%)	
Resistant to LVFX	44 (83.0%)	
Resistant to STFX	10 (18.9%)	
Resistant to MTX	47 (88.7%)	
Type of regimen	Patient, No.	Eradication rates, n (%)
Total patient	53	42 (79.2%)
VPZ, AMX, STFX for 1wk	29	26 (89.7%)
PPI, AMX, STFX for 1wk	14	9 (64.3%)
VPZ, STFX, MINO for 1wk	3	2 (66.7%)
PPI, MTX, MINO for 1wk	2	1 (50.0%)
VPZ, STFX, CAM for 1wk	2	2 (100%)
VPZ, AMX, MINO for 1wk	1	1 (100%)
VPZ, AMX, STFX, MTX for 1wk	1	1 (100%)
PPI, AMX, MTX, MINO for 1wk	1	0 (0%)

AMX, amoxicillin; CAM, clarithromycin; LVFX, levofloxacin; STFX, sitafloxacin; MTX, metronidazole; VPZ, vonoprazan; PPI, proton pump inhibitor; MINO, minocycline.

female, 14 had a history of smoking, 20 did not smoke, and 19 had an unknown history. Antibiotic susceptibility test results indicated that nine patients (17.0%) were not sensitive to AMX. Resistance to CAM was observed in 51 patients (96.2%), whereas that to LVFX was observed in 44 patients (83.0%). Additionally, resistance to STFX and MTX was observed in 10 (18.9%) and 47 patients (88.7%), respectively.

Type of regimen selected and success rate of third-line eradication

The overall eradication rate for third-line therapy was 79.2% (42/53 patients). The eradication rates by type of regimen at 1 week were 26/29 (89.7%) for VPZ, AMX, and STFX; 9/14 (64.3%) for PPI, AMX, and STFX; 2/3 (66.7%) for VPZ, STFX, and MINO; 1/2 (50%) for PPI, MTX, and MINO; 2/2 (100%) for VPZ, STFX, and CAM; 1/1 (100%) for VPZ, AMX, and MINO; 1/1 (100%) for VPZ, AMX, STFX, and MTX; and 0/0 (0%) for PPI, AMX, MTX, and MINO (Table 1).

Comparison of clinical characteristics between third-line eradication success and failure groups using regimens containing AMX and STFX

Table 2 compares the clinical characteristics of patients in the eradication success and failure groups treated with regimens containing PPI, VPZ, AMX, and STFX. The success group comprised 35 patients, whereas the failure group included 8 patients. The median age was 50 (IQR, 44–63) years in the eradication success group and 56 (IQR, 46–62) years in the eradication failure group, with no significant differences (p=0.512). Eradication was successful in six smokers (54.5%) and 14 non-smokers (93.3%), respectively, with no significant difference between the two groups (p=0.054). Eradication was successful in 27 females (96.5%) and 8 males (53.3%). The eradication success rate was significantly higher in women than males (p=0.001). Of the 15 male patients, 4 (26.7%) were resistant to AMX, and 5 (33.3%) were resistant to STFX. In contrast, among the 28 female patients, 4 (14.3%) were resistant to AMX, and 2 (7.1%) were resistant to STFX. Eradication was successful in 9 patients (64.3%) treated with PPI and in 26 patients (89.7%) treated with VPZ, with no significant difference between both groups (p=0.089). Among patients sensitive to AMX, 31 (88.6%) achieved successful eradication, compared to four patients (50%) who were not sensitive to AMX. Sensitivity to AMX was associated with a significantly higher eradication success rate (P=0.028). Among patients sensitive to STFX, 31 (86.1%) achieved successful eradication compared to four patients (57.1%) who were resistant to STFX, with no significant difference between both groups (p=0.106). The eradication success rate was 77.1% (27 cases) among patients sensitive to both AMX and STFX, 22.9% (8 cases) among patients resistant to either, and 0% (0 cases) among patients resistant to both drugs, with a significant difference (p=0.014).

Discussion

A regimen comprising VPZ, AMX, and STFX is considered a good option for the third-line eradication of *H. pylori* in Japan. The overall eradication rate for third-line eradication, including in the period before the launch of VPZ in this study, was 79.2%, which was lower than that reported in previous studies [5]. However, the eradication rate for the most frequently selected VPZ,

Table 2. Comparison of Factors Related to Patients and Lesions between the Success and Failure Groups of Patients in whom *Helicobacter pylori* was Eradicated Using AMX and STFX Regimens

	Success group	Failure group	p-value
Patient, No.	35	8	
Age, year (median [IQR])	50 (44–63)	56 (46–62)	0.512†
Sex, n (%)			
Male	8 (22.9%)	7 (87.5%)	0.001*
Female	27 (77.1%)	1 (12.5%)	
Smoking, n (%)			
+	6 (30.0%)	5 (83.3%)	0.054*
−	14 (70.0%)	1 (16.7%)	
Anti-acid drug, n (%)			
PPI	9 (25.7%)	5 (62.5%)	0.089*
VPZ	26 (74.3%)	3 (37.5%)	
Antibiotic susceptibility test, n (%)			
Sensitive to AMX	31 (88.6%)	4 (50.0%)	0.028*
Not sensitive to AMX	4 (11.4%)	4 (50.0%)	
Sensitive to STFX	31 (88.6%)	5 (62.5%)	0.106*
Resistant to STFX	4 (11.4%)	3 (37.5%)	
Sensitive to AMX and STFX	27 (77.1%)	3 (37.5%)	0.014*
Resistant to AMX or STFX	8 (22.9%)	3 (37.5%)	
Resistant to AMX and STFX	0 (0%)	2 (25%)	

PPI, proton pump inhibitor; VPZ, vonoprazan; AMX, amoxicillin; and STFX, sitafloxacin; * Fisher's exact test; † Mann–Whitney U test

AMX, and STFX regimen was 89.7%, comparable to previously reported high rates [2-4].

The characteristics associated with successful third-line eradication were female sex and AMX sensitivity, both of which showed significant differences. Females may have more opportunities to take antibiotics and MTX for urinary and gynecological infections [10], leading to higher rates of MTX [11]. The proportion of female patients was higher in the study group. Additionally, resistance to AMX and STFX in this patient group was higher in males than in females, which may have contributed to the higher eradication rates observed in female patients.

Although not significant, VPZ use was associated with higher eradication rates. Compared to PPI, VPZ provides stable acid suppression, independent of CYP2C19 genetic polymorphisms [11]. *H. pylori* is in the proliferative phase at higher pH and is more sensitive to antibiotics such as AMX; inhibition of high gastric acid secretion also enhances the stability of AMX by preventing its acid degradation [11, 12]. The eradication rate tended to decrease as the number of antibiotic-resistant isolates increased, indicating that antibiotic resistance may lead to eradication failure. Although the sample size was small, 7/10 patients achieved successful eradication with regimens other than AMX and STFX, suggesting that personalized treatment based on antibiotic susceptibility may be effective. Non-smokers have been reported to show higher eradication success rates, and similar trends were observed in this study [13]. However, the retrospective nature of this study and inability to ascertain smoking history in many cases limited a detailed analysis of smoking history and eradication rates.

This study has several limitations. First, as a single-center retrospective study including only patients with culturable *H. pylori*, selection bias may have occurred. However, as third-line eradication cases account for only 2–3% of all *H. pylori* eradication cases, the collection of 53 cases over seven years is considered meaningful compared to previous reports [2-5, 7]. Second, limited information in medical records, including relapse, reinfection, adverse events, and patient backgrounds, restricted detailed evaluation of tolerability and long-term outcomes. This may affect the interpretation of results. Third, antibiotic resistance was assessed solely through phenotypic susceptibility testing, as genotypic analyses such as gyrA mutations were not performed. Fourth, treatment regimens were selected at the physician's discretion based on available susceptibility data in Japan, limiting the diversity of antibiotics used. This may reduce the generalizability of findings to other settings with different prescribing practices. Future multicenter studies including genetic data and long-term clinical outcomes are warranted to further optimize third-line *H. pylori* eradication strategies.

In conclusion, the VPZ, AMX, and STFX regimens are useful for third-line eradication, with significantly higher eradication success rates observed in female patients and those sensitive to AMX. To expand the options for regimens, antibiotic susceptibility testing should be conducted in all cases [14]. However, performing these tests in all institutions may be challenging. In cases where antibiotic susceptibility testing cannot be performed, the empirical selection of the VPZ, AMX, and STFX regimens for third-line eradication is useful, and high eradication success rates can be expected in female patients.

Author Contribution Statement

KS contributed the conceptualization of the study and drafting of the manuscript. AF contributed to the conceptualization of the study, drafting of the manuscript, and critical revision of the manuscript for intellectual content. HM, YI, TM contributed to the critical revision of the manuscript for important intellectual content. All authors provided final approval of the article.

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Scientific Body Approval / Student Thesis

This study is part of a doctoral thesis to be submitted for the degree of Doctor of Medicine at Toho University.

Data Availability

The data are not available for public access owing to patient privacy concerns but are available from the corresponding author upon reasonable request.

Ethical Declaration

The study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol, including the opt-out informed consent procedure for the use of participant data for research purposes, was approved by the Ethics Committee of Toho University Omori Medical Center (approval number: M21207, date of decision: 3 December 2021).

Conflict of Interest

The authors have no conflicts of interest to declare.

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