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Efficacy of levofloxacin-based rescue therapy for *Helicobacter pylori* infection after standard triple therapy: a randomized controlled trial

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Objectives: This prospective study was designed to determine the efficacy of a levofloxacin-based rescue therapy for *Helicobacter pylori* infection after failure of standard triple therapies. We also surveyed the predictors of this rescue therapy.

Patients and methods: From June 2005 to March 2007, 1036 patients infected with *H. pylori* received standard triple regimens (proton pump inhibitor, clarithromycin and amoxicillin). *H. pylori* eradication was achieved in 855 (82.5%) subjects. One hundred and sixty-six eradication-failure patients were enrolled and randomly assigned to receive a 7 day eradication therapy with esomeprazole, bismuth subcitrate, tetracycline and metronidazole (EBTM) or esomeprazole, amoxicillin and levofloxacin (EAL). Follow-up endoscopy was done 16 weeks later to assess the treatment response. Patients' response, *CYP2C19* genotypes and antibiotic resistances were also examined.

Results: Intention-to-treat analysis revealed that both groups showed similar eradication rates [EBTM 63.9%; 95% confidence interval (CI): 53.6–74.2 and EAL 69.9%; 95% CI: 60.1–79.7] ($P = 0.89$). Per-protocol results were EBTM = 84.1% (95% CI: 75.1–93.1) and EAL = 75.3% (95% CI: 65.8–84.8) ($P = 0.82$). Both regimens had similar compliance ($P = 0.32$), but the EBTM group had more adverse events ($P = 0.27$). Logistic regression analysis showed that poor compliance, CYP2C19 homozygous extensive metabolizer genotype and levofloxacin resistance were important predictors for eradication failure.

Conclusions: The EAL regimen can achieve an efficacy similar to that of the standard EBTM therapy. It may be very useful in countries where bismuth salts are not available. Compliance, CYP2C19 genotype and resistances to antibiotics may influence the outcome of levofloxacin-based rescue therapy. It seems advisable to reserve levofloxacin for rescue treatment to avoid an increase in the resistance phenomenon.

Keywords: fluoroquinolones, *H. pylori*, CYP2C19

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Introduction

Helicobacter pylori infection is known to be associated with the development of gastritis, peptic ulcer and gastric cancer.[1] The worldwide prevalence of *H. pylori* infection is ∼50%, with the highest being in developing countries. In Taiwan, the overall prevalence rate is 54% and this rises with age.[2] Therefore, it is important to develop and evaluate different treatment regimens. Seven day triple therapy [proton pump inhibitor (PPI), amoxicillin and clarithromycin] has been the main first-line therapy for *H. pylori* infection in Taiwan, Europe and many other countries in accordance with the Maastricht-2 2000 Consensus.[3,4] In Taiwan, an endemic area of *H. pylori* infection with high metronidazole resistance, the regimen using a combination of PPI with amoxicillin and clarithromycin has been shown to be better than the other regimens of PPI-based triple therapy.[5] Despite this, such a widely recommended first-line regimen continued to have a 10% to 23% failure rate.[5–10] With the increase in antibiotic resistance, initial triple therapy has become less efficacious. Recent studies have shown that the average cure rate is ∼70% to 89%.[11] Resistance of *H. pylori* to clarithromycin is an important reason for treatment failure.[12,13]

When administering a second-line treatment for *H. pylori* infection, it is important to choose adequate antibiotics that do not face resistance problems.[14] Bismuth salts are often applied to decrease bacterial load.[15] Currently, the most widely used second-line therapy is quadruple therapy, consisting of a PPI, a bismuth salt, metronidazole and tetracycline.[3,4,16] However, several studies have shown that this rescue regimen has failed in 5% to 63% of patients, whose *H. pylori* cannot be eradicated by standard PPI-based triple therapies.[17,18] The complex drug regimen and side effects of quadruple therapy may have poor compliance in patients and thus decrease its efficacy. Moreover, the standard quadruple therapy (PPI, bismuth salts, tetracycline and metronidazole) is not feasible in some countries; bismuth compounds being unavailable. The data indicate that it is still essential to search for an optimal second-line regimen to eradicate *H. pylori* with consistent and high efficacy.

Levofloxacin is a fluoroquinolone that exerts bactericidal effects by the inhibition of the DNA gyrase.[19] It is a broad-spectrum fluoroquinolone, active against Gram-positive and -negative bacteria and atypical respiratory pathogens.[20] Fluoroquinolones are active against *H. pylori in vitro*[21] and have a synergistic effect with PPIs.[22] The currently reported per-protocol (PP) efficacy of levofloxacin-based therapy has a wide range, from 60% to 90%.[18,23–26] Therefore, the precise effective dosage of levofloxacin still needs to be determined.

As known, many antibiotics used for eradicating *H. pylori* are acid-sensitive. PPIs not only increase the activity of some antibiotics by reducing gastric acid secretion but also possess direct anti-*H. pylori* activity.[27,28] They are metabolized by the hepatic cytochrome P450 system, especially *S*-mephenytoin 4′-hydroxylase (CYP2C19).[29] There are genetically determined differences in the activity of these enzymes, leading to variable plasma PPI levels and intragastric pH during PPI treatment.

We therefore designed a prospective study to compare the efficacies of standard quadruple therapy and a new second-line therapy consisting of a PPI (esomeprazole), amoxicillin and levofloxacin for the treatment of patients after failure of standard first-line therapy. Furthermore, we also investigated the impact of antibiotic resistance and polymorphism of CYP2C19 on the eradication rates of these two second-line therapies.

Patients and methods

Participants

Patients were those who visited the gastroenterological clinic of Kaohsiung Medical University Hospital (KMUH) and Kaohsiung Veterans General Hospital (KSVGH) between June 2005 and March 2007 with the complaint of dyspepsia. *H. pylori*-infected patients received first-line eradication therapies with standard triple regimens (PPI twice daily, 500 mg of clarithromycin twice daily and 1 g of amoxicillin twice daily). Then eradication-failure patients were enrolled for this study after giving informed consent. The presence of *H. pylori* after a previous eradication therapy was defined as: (i) at least two positive results of rapid urease test, histology and ^{13}C-urea breath test (UBT); or (ii) a positive result of culture. Exclusion criteria included: (i) ingestion of antibiotics, bismuth or PPI within the prior 4 weeks; (ii) patients with allergic history to the medications used; (iii) patients with previous gastric surgery; (iv) the coexistence of serious concomitant illness (e.g. decompensated liver cirrhosis, uraemia); and (v) pregnant women. All of the participants underwent a ^{13}C-UBT and endoscopic examination with biopsy of the gastric mucosa to establish *H. pylori* infection status.

Interventions

We included 166 cases (84 men and 82 women; mean age: 49.8 ± 12.7 years, range: 16–74) that met eradication failure of *H. pylori* infection. A trained interviewer who used a standardized questionnaire to obtain demographic data and medical history interviewed them. The participants were randomly assigned to the EBTM group (40 mg of esomeprazole twice daily, 120 mg of bismuth subcitrate four times daily, 500 mg of tetracycline four times daily and 250 mg of metronidazole four times daily for 7 days) or the EAL group (40 mg esomeprazole twice daily, 1 g of amoxicillin twice daily and 500 mg of levofloxacin once daily for 7 days). Patients were asked to return during the second week to assess drug compliance and adverse effects. Endoscopy with biopsy for rapid urease test, histology and culture was repeated 16 weeks later to confirm *H. pylori* infection status. For patients who refused follow-up endoscopy, UBT was used to confirm *H. pylori* status. The technicians who performed the *H. pylori* tests (culture, rapid urease test and UBT) or filled in the questionnaires as well as the pathologists were blinded to the eradication regimens the patients received. All participants gave written informed consent. This study was approved by both the Institutional Review Board and the Ethics Committee of KMUH and KSVGH. The results of this study were reviewed every 2 months to monitor the possible ethical problems.

Objectives

In the current study, we tested the hypothesis that the levofloxacin-based rescue therapy would be safer and more effective than the bismuth-containing standard second-line therapy. Other hypotheses involved testing that antibiotic resistances and CYP2C19 genotypes would influence the outcome of rescue therapy.

Outcomes

The primary endpoint of our study was successful eradication of *H. pylori*. There were additional analyses on adverse events during therapies.

Questionnaire

The questionnaire contained questions regarding personal history of smoking and drinking alcohol. Smokers were those who consumed more than 1 pack of cigarettes a week and drinkers were those who drank more than 1 cup of alcoholic beverage a day. Compliance was defined as good (>70% of the total medication taken) or poor by counting unused medication after the treatment was completed. The adverse events included abdominal pain, diarrhoea, constipation, dizziness, taste perversion, headache, anorexia, nausea, vomiting and skin rash. Those who considered that those symptoms disturbed their daily life were defined to have positive adverse effects. Those who did not experience these symptoms were defined as negative adverse effects. Well-trained assistants were used to complete the questionnaire designed in the study.

Diagnosis of H. pylori infection

Culture and pathological examination. Biopsy specimens were rubbed on the surface of a Columbia blood agar plate and then incubated at 35°C under microaerobic conditions for 4–5 days. The result for the Gram's stain was considered positive when a curvy, Gram-negative bacterium was found on the smear. Culture of *H. pylori* was considered positive if one or more colonies showed Gram-negativity, positive oxidase, catalase and urease tests and spiral or curved bacilli. The biopsy specimens were fixed with formalin, embedded in paraffin and stained with haematoxylin and eosin. They were interpreted and reported on by the same pathologist. This method provided additional information about gastric mucosal changes, including atrophy, dysplasia, metaplasia and the pattern and degree of inflammation.

Rapid urease test. The results of the rapid urease test (CLO test; Delta West Bentley, WA Australia) were interpreted as positive if the colour of the gel turned pink or red 6 h after examination at room temperature.

^{13}*C-UBT.* The ^{13}C-urea was manufactured by the Institute of Nuclear Energy Research, Taiwan. One hundred millilitres of fresh whole milk was used as the test meal. This detailed procedure was reported in our previous study.[30] For patients who received follow-up endoscopy, *H. pylori* infection was established if the culture was positive, or both CLO test and histology were positive.

Culture and antimicrobial resistance

One antral gastric biopsy specimen was obtained for the isolation of *H. pylori*, using previously described culture methods.[31] *H. pylori* culture was performed by rubbing the specimens on the surface of a Campy-BAP agar plate [Brucella agar (Difco, Sparks, MD, USA)+IsoVitalex (Gibco, Grand Island, NY, USA)+10% whole sheep blood]. Then, they were incubated at 37°C under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2) for 4–5 days. The results were considered positive if one or more colonies of Gram-negative bacilli with positive oxidase, catalase and urease tests were found. The *H. pylori* strains were tested for tetracycline, metronidazole, amoxicillin and levofloxacin susceptibility using the Etest (AB Biodisk, Solna, Sweden). *H. pylori* strains with MICs >4, >8, >0.5 and >1 mg/L were considered to be resistant to tetracycline, metronidazole, amoxicillin and levofloxacin, respectively.

Analysis of CYP2C19 genotypes

For the analysis of *CYP2C19* genotypes, all enrolled patients' peripheral blood leucocytes were obtained before the eradication therapy was begun. DNA was extracted from the leucocytes with a commercially available kit (Qiagen K.K., Tokyo, Japan) and stored until use. Genotyping procedures for identifying the *CYP2C19 wild-type* (*wt*) gene and two mutated alleles, *CYP2C19 m1* and *CYP2C19 m2*, were performed by a PCR–restriction fragment length polymorphism method with allele-specific primers.[32,33]

Randomization

A computer-generated randomization list was used to generate a 'random sequence'. We used a method combining blocking and stratified randomization to ensure a close balance of the numbers and patients' characteristics in each group. We set separate randomization within each of two subsets of participants (age and sex). We also set a block of every 10 participants. A computer-generated randomization list was drawn up by the statistician and given to our assistant member responsible for randomization. Doctors determined patients' suitability to be enrolled in this study and allocated the next available number on entry into the trial. Each patient collected his/her tablets directly from the pharmacy department. The code was revealed to the researchers once recruitment, data collection and laboratory analyses were complete. All study participants and doctors except the data monitoring committee were blinded to treatment assignment for the duration of this study. The data monitoring committee did not have contact with participants. During this period, to evaluate the success of blinding, we administered a questionnaire twice to ask participants which treatment they thought they had received (real drug, placebo or unknown). In the questionnaire, their reasons were also recorded to evaluate the success of the blinding procedure.

Statistical analysis

Design. Assuming that the conventional eradication rate (in the EBTM group) was 70% and that the EAL group achieved a 90% eradication rate, a 20% increase, our statistical power in this study will be 90% with sample sizes of about 80 subjects in each group and have a two-sided *P* value of 0.05 if 95% of patients completed the follow-up.

Data analyses. The distribution of gender and the initial endoscopic diagnosis between subjects in the EBTM and EAL groups were compared by χ^2 statistics. The same method was applied to compare the efficacy and the frequency of the side effects of the two regimens. The analysed efficacy outcome was cure of *H. pylori* infection. The difference in patients' ages in the two groups was examined using Student's *t*-test. A two-sided *P* value of <0.05 was considered statistically significant. The data were analysed using the SAS statistical package; all *P* values were two-sided.

Eradication rates were evaluated by intention-to-treat (ITT) and PP analyses. ITT analysis included all randomized patients. Patients whose infection status was unknown following treatment were considered treatment failures for the purposes of ITT analysis. The PP analysis excluded patients with unknown *H. pylori* status following therapy and those with major protocol violations. A *P* value of <0.05 was considered statistically significant. To determine the predictors affecting the treatment response, clinical and bacterial parameters were analysed by univariate analysis. Those predictors found to be significant by univariate analysis were subsequently assessed by a stepwise logistic regression method to identify independent factors for eradication outcome. The 95% confidence intervals (CIs) for the relative risks were also calculated. Significance levels were determined with the use of two-tailed tests.

Kuo _et al._

Table 1. Demographic distribution of the subjects receiving different eradication regimens

	EBTM	EAL	_P_ value
No. of patients	83	83	
Male/female	40/43	44/39	0.45
Age (years), mean ± SD	49.1 ± 13.6	50.2 ± 12.4	0.15
Diagnosis			0.41
gastric ulcer	21	19	
duodenal ulcer	33	34	
gastritis	29	30	
Smoker	10	12	0.13

EBTM, esomeprazole, bismuth subcitrate, tetracycline and metronidazole; EAL, esomeprazole, amoxicillin and levofloxacin.

Results

Characteristics of the study groups

One thousand and thirty-six patients infected with _H. pylori_ received first-line eradication therapies with standard triple regimens. _H. pylori_ eradication was achieved in 855 (82.5%) subjects. There were 15 patients who withdrew their consent after enrollment and randomization but before receiving any study medication. A total of 166 _H. pylori_-infected patients were enrolled in our study and randomly assigned to EBTM ($n=83$) or EAL ($n=83$) therapies. The subjects were all included in the ITT analysis for _H. pylori_ eradication. The clinical characteristics of patients at entry are summarized in Table 1. The two groups had comparable age, gender, history of smoking and endoscopic findings. Among the subjects, six with poor compliance and five lost to follow-up were excluded from PP analysis for _H. pylori_ eradication. Both regimens had similar compliance (EBTM=92.9% and EAL=99.5%) ($P=0.32$). Figure 1 summarizes the patient disposition according to the CONSORT statement advice.[34]

Eradication of H. pylori

Table 2 lists the eradication rates of the EBTM and EAL groups. ITT analysis demonstrates similar eradication rates in the two study groups (EBTM 63.9%, 95% CI: 53.6–74.2 and EAL 69.9%, 95% CI: 60.1–79.7) ($P=0.89$). According to the PP analysis, the success rates of eradication _H. pylori_ infection were EBTM=84.1% (95% CI: 75.1–93.1) and EAL=75.3% (95% CI: 65.8–84.8) ($P=0.82$). The eradication rates were similar between groups ($P=0.38$).

Factors influencing efficacy of anti-H. pylori therapy

Table 3 lists the clinical and bacterial factors that might predict the efficacy of eradication therapy. In univariate analyses, the eradication rates were significantly related to drug compliance ($P=0.01$). Resistances to antibiotics were also important factors for successful eradication ($P<0.05$). Besides these, the genotype of CYP2C19 was a significantly influential factor ($P=0.003$). There were no other factors found to influence the eradication efficacy significantly in univariate analysis (data not shown in

table). The logistic regression model was then used to estimate the relative risk of eradication failure associated with drug compliance, resistance to antibiotics and CYP2C19 genotypes. The relative risk ratio of drug compliance was 2.37 (95% CI: 1.22–4.16). The relative risk ratio of the CYP2C19 homozygous (hom) extensive metabolizer (EM) genotype was 1.75 (95% CI: 1.87–17.72). The relative risk ratio of metronidazole resistance was 0.66 (95% CI: 0.58–6.39). The relative risk ratio of amoxicillin resistance was 1.59 (95% CI: 0.58–3.64). The relative risk ratio of levofloxacin resistance was 1.51 (95% CI: 1.28–16.05). It disclosed that poor compliance, CYP2C19 hom EM and resistance to levofloxacin were independent factors predictive of treatment success.

Antibiotic resistance

H. pylori strains were isolated from 99 of all enrolled patients who underwent bacterial culture at initial endoscopy. No strains developed resistance to tetracycline. Metronidazole-, amoxicillin- and levofloxacin-resistant strains were found in 56.6% (56/99), 6.1% (6/99) and 21.2% (21/99) of the patients, respectively. According to univariate analysis, the resistances to these antibiotics all influenced the outcome of _H. pylori_ eradication ($P<0.05$) (Table 3).

Genotypes of CYP2C19

For _CYP2C19_ genetic polymorphism, six different allelic patterns were noted: _wt/wt_, _wt/m1_, _wt/m2_, _m1/m1_, _m1/m2_ and _m2/m2_. Patients were classified into three groups according to the genotype: (i) those without mutation (_wt/wt_; $n=54$, 35.8%), designated as the hom EM group; (ii) those with one mutation (_wt/m1_ or _wt/m2_; $n=76$, 50.3%), designated as the heterozygous EM (het EM) group; and (iii) those with two mutations (_m1/m1_ or _m1/m2_ or _m2/m2_; $n=21$, 13.9%), designated as the poor metabolizer (PM) group. We show the cure rate according to the _CYP2C19_ genotype in Figure 2. A significantly poor cure rate was observed in the _CYP2C19_ hom EM group (_CYP2C19_ hom EM versus _CYP2C19_ het EM+PM, $P=0.03$).

Proportion of CYP2C19 genotypes among different outcomes of eradication

The proportion of different genotypes of CYP2C19 is shown in Figure 2. The incidence of hom EM and het EM genotypes was slightly higher in the group with failure of eradication than in the group with success of eradication, but the difference was not significant. While the incidence of the PM genotype was significantly lower in the group with eradication failure than in the group with successful eradication, no PM genotype was found with failure of eradication in the EBTM group.

Adverse events and complications

Interviews regarding adverse events were carried out in all patients. Adverse events were reported in 35 (43.6%) of the 151 patients (Table 4). 35.2% (25/71) of the EBTM group and 12.6% of the EAL group reported at least one adverse event. Adverse events during eradication did not result in significantly different compliance between the two groups ($P=0.78$). Nausea was the
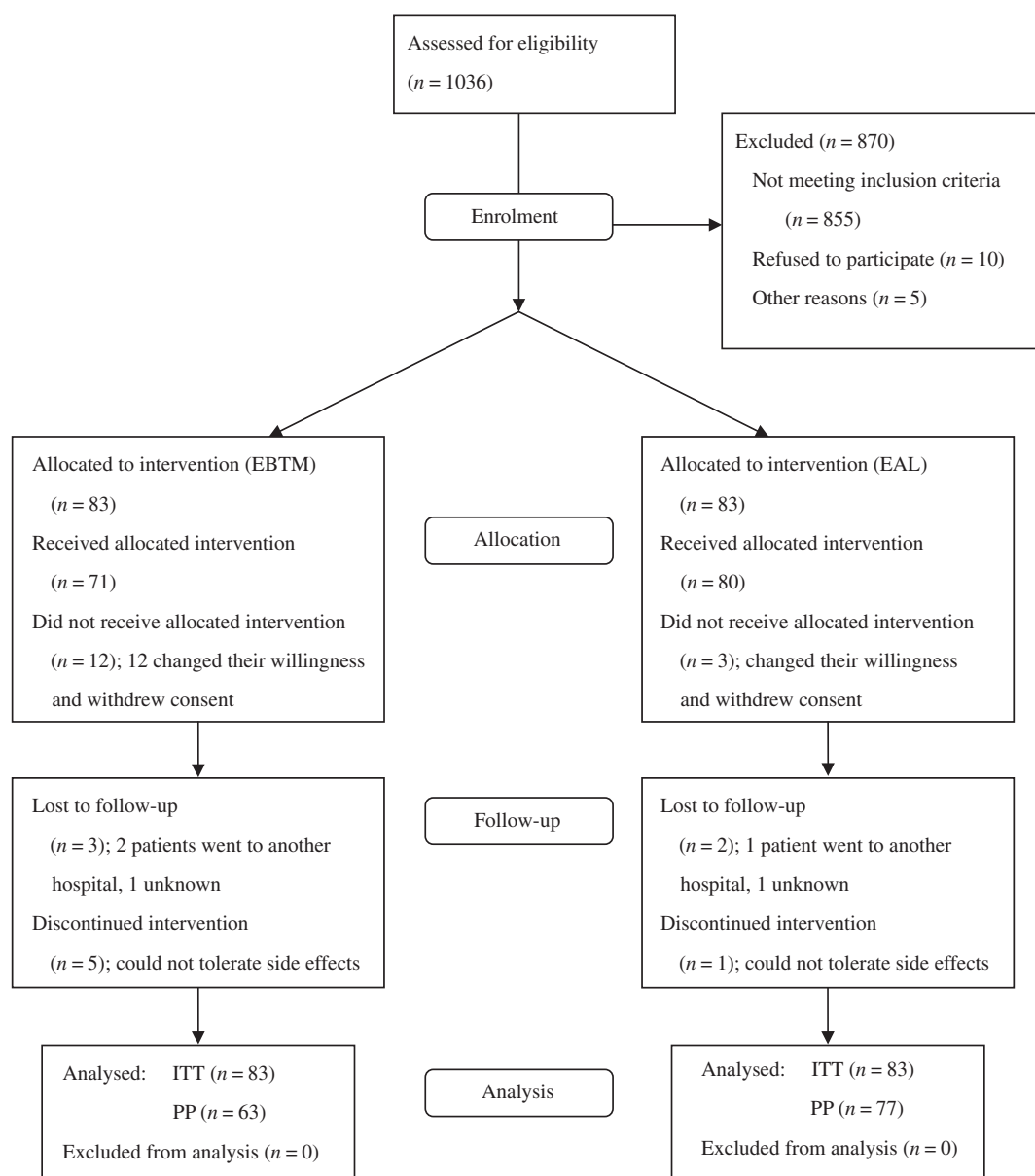
Efficacy of levofloxacin-based rescue therapy

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                    ┌─────────────────────────────┐
                    │ Assessed for eligibility    │
                    │ (n = 1036)                  │
                    └─────────────────────────────┘

                                                    ┌──────────────────────────────────────┐
                                                    │ Excluded (n = 870)                     │
                    ┌──────────────┐                │   Not meeting inclusion criteria       │
                    │  Enrolment   │──────────────► │     (n = 855)                          │
                    └──────────────┘                │   Refused to participate (n = 10)      │
                                                    │   Other reasons (n = 5)                │
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Allocated to intervention (EBTM)		Allocated to intervention (EAL)
(n = 83)	**Allocation**	(n = 83)
Received allocated intervention		Received allocated intervention
(n = 71)		(n = 80)
Did not receive allocated intervention		Did not receive allocated intervention
(n = 12); 12 changed their willingness and withdrew consent		(n = 3); changed their willingness and withdrew consent

Lost to follow-up		Lost to follow-up
(n = 3); 2 patients went to another hospital, 1 unknown	**Follow-up**	(n = 2); 1 patient went to another hospital, 1 unknown
Discontinued intervention		Discontinued intervention
(n = 5); could not tolerate side effects		(n = 1); could not tolerate side effects

Analysed: ITT (n = 83)		Analysed: ITT (n = 83)
PP (n = 63)	**Analysis**	PP (n = 77)
Excluded from analysis (n = 0)		Excluded from analysis (n = 0)

Figure 1. Disposition of patients. ITT, intention-to-treat; PP, per-protocol; EBTM, esomeprazole, bismuth subcitrate, tetracycline and metronidazole; EAL, esomeprazole, amoxicillin and levofloxacin.

most common adverse event. In the EBTM group, two patients discontinued treatment because of skin rash. In the EAL group, there was one patient who stopped the eradication due to severe headache. Altogether, six patients discontinued the treatment due to adverse events. Both groups displayed similar compliance rates (EBTM 93% and EAL 97.5%, $P=0.68$).

Discussion

The *H. pylori* eradication rate following triple therapies has substantially decreased in the last 5 years. Therefore, a continuous search for novel therapeutic approaches to cure *H. pylori* infection is needed.[35] In the present study, we have further tested the efficacy of these promising therapy regimens for *H. pylori* eradication.

In our study, both regimens showed similar efficacy and safety. They showed acceptable eradication rates (ITT: 63.9% and 69.9% for EBTM and EAL, respectively) (PP: 84.1% and 75.3% for EBTM and EAL, respectively). They also showed a high cumulative eradication rate (96.3%) (data not shown).[36] Our data showed that levofloxacin-based rescue therapy was a useful regimen.

Levofloxacin inhibits DNA synthesis, has a good oral absorption and is well tolerated.[37] The levofloxacin-based triple therapy was simple and well tolerated in the present study. Our data also showed high compliance (97.5%) and a relatively lower adverse event rate compared with the bismuth-based regimen (12.5% versus 35.2%). Compliance plays a cardinal role in eradication.

The common side effects of PPI-based therapy include abdominal symptoms (e.g. abdominal pain, diarrhoea, constipation and

Table 2. Outcomes of EBTM and EAL rescue therapies

	EBTM group (n=83)	EAL group (n=83)	P value
Eradication rate			
intention-to-treat	63.9% (53/83)	69.9% (58/83)	0.89
per-protocol	84.1% (53/63)	75.3% (58/77)	0.82
Compliance	92.9% (66/71)	99.5% (79/80)	0.32
Adverse events	35.2% (25/71)	12.5% (10/80)	0.27

EBTM, esomeprazole, bismuth subcitrate, tetracycline and metronidazole; EAL, esomeprazole, amoxicillin and levofloxacin.
In this analysis, patients with unknown outcome are counted as treatment failures.

Table 3. Logistic regression model analysis of the clinical factors influencing the efficacy of *H. pylori* eradication therapy

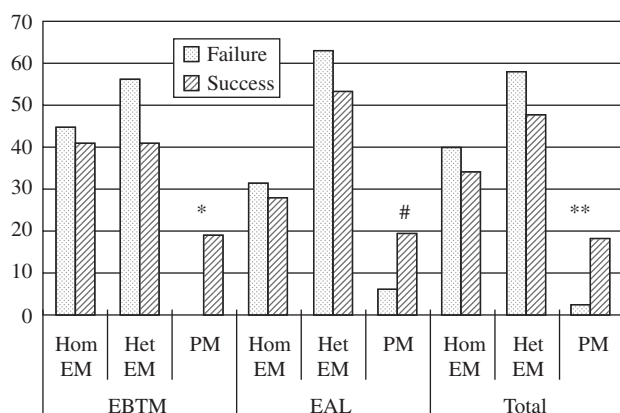| Parameter	Risk ratio	95% CI (lower)	95% CI (upper)
Poor compliance	2.37	1.22	4.16
CYP2C19 genotype (hom EM)	1.75	1.87	17.72
Resistance to metronidazole	0.66	0.58	6.39
Resistance to levofloxacin	1.51	1.28	16.05
Resistance to amoxicillin	1.59	0.58	3.64

EM, extensive metabolizer.

Figure 2. Proportion of CYP2C19 genotypes in different patient groups. The poor metabolizer genotype of CYP2C19 showed a significantly higher proportion in successful groups compared with failed groups in each regimen and total patients. *$P=0.03$, **$P=0.02$, #$P=0.02$. EBTM, esomeprazole, bismuth subcitrate, tetracycline and metronidazole; EAL, esomeprazole, amoxicillin and levofloxacin.

nausea, skin rash, headache and dizziness). In our study, nausea was the most common adverse effect complained about. Most adverse events were self-limited.

Primary resistance to levofloxacin ranges between 8% and 31% in different countries or regions.[37–39] In our data, the resistance rate was 21.2%, and it was the important factor that influenced the outcome of eradication.

Table 4. Adverse events of EBTM and EAL rescue therapies

Adverse events	EBTM (n)	EAL (n)
Abdominal pain	3	3
Diarrhoea	2	0
Constipation	1	0
Headache	3	3
Anorexia	5	1
Nausea	12	1
Vomiting	5	1
Skin rash	1	0
Others	19	10

EBTM, esomeprazole, bismuth subcitrate, tetracycline and metronidazole; EAL, esomeprazole, amoxicillin and levofloxacin.
The numbers shown are patients who suffered from mild, moderate and severe adverse events.

The levofloxacin-based treatment could eradicate most of the strains (92.3%) that were resistant *in vitro* to both clarithromycin and metronidazole, but were susceptible to levofloxacin. Furthermore, this drug combination is well tolerated and has no major side effects.[40] However, resistance to quinolones is easily acquired. The use of levofloxacin should be confined to 'rescue' therapy, in order to avoid rapidly increasing *H. pylori* resistance towards such an antibiotic.[26] Indeed, primary levofloxacin resistance is increasing, with values of 5.5% to 14.3% in Japan,[41–43] 17% in Brazil,[25] 18% in Hong Kong,[18] 21.5% in Korea[44] and 9% to 32.3% in Italy.[37,45]

There were many different results with bismuth-based and levofloxacin-based regimens reported in previous studies.[18,46] There must be some factors other than resistance and compliance that influence the eradication results. PPIs are affected by the CYP2C19 polymorphism to variable degrees. It would result in different inhibitory degrees of gastric acid, because most antibiotics used for eradicating *H. pylori* are acid-sensitive, so the genotype of CYP2C19 would have an effect on eradication. In our study, results of both univariate and logistic regression analyses supported this concept. We demonstrated that the successful eradication group had a high proportion of the PM genotype of CYP2C19. Logistic regression analysis disclosed that the CYP2C19 hom EM genotype was an independent factor of the eradication rate in levofloxacin-based rescue therapy. So it may be advisable to survey the genotype of CYP2C19 for refractory *H. pylori* infection.

In our study, we used an esomeprazole-based regimen. Esomeprazole has minimal first-pass metabolism, undergoes less hydroxylation via CYP2C19 and has been shown to have a greater gastric acid suppression effect than omeprazole.[47,48] In further studies, we may use different PPIs to test the impact of CYP2C19 genotypes on rescue therapies.

The dosage of levofloxacin was 500 mg daily in our study. Some previous studies used 1000 mg daily. However, they found that increasing the dosage of levofloxacin cannot overcome levofloxacin resistance.[18,46] In contrast, our study applied levofloxacin for just 7 days. It might be a limitation of our study. In a meta-analysis, it has been reported that a shorter treatment may be relatively less effective for *H. pylori* eradication.[49] Therefore, our 7 day therapy should be described as an acceptably effective

regimen, but a longer duration of such therapy may have the effect of an improved eradication. Another limitation of our study was that there were 15 patients who retracted their permission after randomization. So we did not start the treatment on them. We regarded these patients as eradication failures, and it resulted in a lower eradication rate in the ITT analysis.

In conclusion, the levofloxacin-based regimen is a reliable therapy for patients with primary eradication failure. Resistance to levofloxacin, the hom EM genotype of CYP2C19 and poor compliance were important influencing factors on rescue eradication. It seems advisable to reserve levofloxacin for rescue treatment to avoid an increase in the resistance phenomenon.

Transparency declarations

None to declare.

C-H. K. designed the study, wrote the Methods section and analysed the results. H-M. H., F-J. Y. and W-M. W. helped conduct the literature review and wrote the Discussion. A. C., I-C. W. and S-W. W. helped conduct the literature review, obtained informed consents and did personal data collection. C-M. J. had the idea for the study and helped with the literature review. F-C. K. and L-L. C. had the idea for the study and helped with study design. P-Y. T., C-J. L. and B-C. W. assisted in case collection and material preparation. P-I. H. and D-C. W. designed and supervised the study and directed its implementation, including quality assurance and control.

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