Rescue therapy with rifabutin regimen for refractory Helicobacter pylori infection with dual drug-resistant strain

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Research article
Abstract

Background:

There is no current standard rescue treatment for dual drug-resistant strains of *Helicobacter pylori*. This aim of this study was to investigate the efficacy of rifabutin-based triple therapy for patients infected with dual drug-resistant strains to clarithromycin and levofloxacin.

Methods:

After two or three *H. pylori* treatment failures, patients underwent upper endoscopy with tissue biopsies. Phenotypic and genotypic resistance was determined using agar dilution test and polymerase chain reaction with direct sequencing, respectively. Patients infected with dual drug-resistant (clarithromycin and levofloxacin) strains and received rifabutin based triple therapy (rifabutin 150 mg bid, amoxicillin 1 g bid and esomeprazole 40 mg bid for 10 days) were enrolled. Eradication status was determined by 13C-urea breath test four weeks after treatment completion.

Results:

A total of 39 patients infected with dual drug-resistant strains were enrolled in this study, with a mean age of 55.9 years. The eradication rate was 79.5% (31/39). Adverse event was reported in 23.1% (9/39) of patients but mild and tolerable. In univariate analysis, no factor was identified as an independent predictor of eradication failure.

Conclusions:

Our current study demonstrated that rifabutin-based triple therapy was well tolerated and yielded an acceptable eradication rate for patients infected with dual drug-resistant strains of *H. pylori*.

Background

*Helicobacter pylori* is a well-known pathogen associated with several upper gastrointestinal diseases, including peptic ulcer disease, atrophic gastritis and malignancies (gastric cancer and mucosa-associated lymphoid tissue lymphoma) [1, 2]. In areas of low clarithromycin resistance, clarithromycin base triple therapy is recommended as first-line empirical treatment [3]. The successful rate of eradication of *H. pylori* have declined recently, mainly due to increasing prevalence of drug resistance [4, 5]. In regions with high resistance to clarithromycin, the effectiveness of the standard triple therapy is lower than 80% [6]. Levofloxacin-based triple therapy (proton pump inhibitor [PPI], amoxicillin, and levofloxacin) is considered a rescue treatment if one or more prior treatment attempts failed. However, resistance to fluoroquinolones is also emerging, and the prevalence in Europe is now close to 15% [7].

The Maastricht IV/Florence consensus report suggests that culture and antimicrobial sensitivity testing should be performed when designing a treatment strategy after one or two treatment failures with
different antibiotics [3].

Rifabutin, which mostly used against *Mycobacterium tuberculosis* and *Mycobacterium avium intracellulare* infection, has been applied as alternative regimen for *H. pylori* eradication [8, 9, 10]. The aim of the present study was to evaluate the efficacy of rifabutin regimen in patients infected with dual strains resistant to clarithromycin and levofloxacin.

**Methods**

**Study design**

After two or three times *H. pylori* eradication failure, patients underwent upper gastrointestinal endoscopy with biopsy of the stomach mucosa for subsequent bacterial culture and molecular test for drug resistance. Phenotypic and genotypic resistance was determined using agar dilution test and polymerase chain reaction (PCR) with direct sequencing, respectively. Only patients infected with *H. pylori* strains harboring dual resistance to clarithromycin and levofloxacin but sensitive to amoxicillin were enrolled in this study and treated with rifabutin-based therapy (rifabutin 150 mg bid, amoxicillin 1 g bid and esomeprazole 40 mg bid) for 10 days. Eradication status was determined by 13C-urea breath test performed 4 weeks later after treatment completion. Self-reported drug adherence and adverse events were recorded during follow-up visiting. Figure 1 demonstrates the schematic flow chart of the study design. This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (IRB No. 201701000A3).

The primary end point of the study was eradication rate. The secondary end point was the rate of adverse effects. Associated factors for successful eradication were also assessed. Patients who lost 13C-urea breath test follow-up and who with unsuccessful *H pylori* culture were excluded.

**Antibiotic susceptibility**

The specimens of gastric biopsy were incubated at 37.8°C under microaerophilic conditions for 10–14 days. Positive cultures were usually identifiable after 3 to 5 days of incubation. Isolates were identified as *H. pylori* according to colony morphology, Gram staining, and results of urease, catalase, and oxidase tests.

Antibiotic susceptibility was determined by agar dilution test (E-test) of *H. pylori* culture and real-time PCR for DNA sequencing of gastric biopsy specimens. Isolated strains were tested for amoxicillin, clarithromycin, and levofloxacin resistance using break points for minimum inhibitory concentrations of ≥ 0.5, ≥ 1, and > 1 µg/mL, respectively. Point mutations (A2143G, A2142G, and A2142C) in the 23S rRNA gene and point mutations in the DNA gyrase A gene (codons 87 and 91), which are associated with clarithromycin and levofloxacin resistance respectively, were also determined.

**Statistical analysis**
Comparison of the patients’ demographic characteristics, eradication rates, and frequency of adverse events was conducted using Fisher’s exact test and Student’s t-test, as appropriate. Statistical analyses were performed using SPSS version 22 for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as mean±standard deviation.

Results

Baseline demographic and clinical data

A total of 39 patients (males, 16; female, 23; mean age, 55.9 ± 12 years) infected with dual drug-resistant strains (clarithromycin and levofloxacin) were enrolled for analysis. Indications for eradication therapy included peptic ulcer disease (20.5%) and nonulcer dyspepsia (79.5%). Demographic and clinical data are summarized in Table 1.

Table 1
Baseline data of patients included in the study (n = 39)

| Age (year) (mean ± SD) | 55.9 ± 12 |
|------------------------|-----------|
| Sex (male/female)       | 16/23     |
| Smokers/nonsmokers      | 5/34      |
| Endoscopic findings     |           |
| Gastric ulcer           | 6 (15.4%) |
| Duodenal ulcer          | 2 (5.1%)  |
| Nonulcer dyspepsia      | 31 (79.5%)|

Values are presented as mean ± standard deviation, n/n, or n (%).

GERD, gastroesophageal reflux disease.

Eradication rates

Eradication status was determined by $^{13}$C-urea breath test, which was carried out no earlier than 4 weeks and up to 8 weeks after cessation of treatment. The cut-off value for a negative UBT was < 4. Overall, the infection was eradicated in 31 patients, corresponding to a cure rate of 79.5%. In univariate analysis, no factor was identified as an independent predictor of eradication failure (Table 3).
Table 3
Univariate analysis of the clinical factors influencing the efficacy of Helicobacter pylori eradication

| Principle parameter | Number of cases | Eradication rate | p value |
|---------------------|-----------------|------------------|---------|
| age                 |                 |                  |         |
| < 60                | 24              | 83.3%            | 0.45    |
| >=60                | 15              | 73.3%            |         |
| sex                 |                 |                  |         |
| Female              | 23              | 82.6%            | 0.56    |
| Male                | 16              | 75.0%            |         |
| smoking             |                 |                  |         |
| (l)                 | 5               | 80.0%            | 0.98    |
| (-)                 | 34              | 79.4%            |         |

Adverse effects

Only mild and tolerable adverse events were reported in 23.1% of patients (Table 2) and anorexia was the most common one. There was no serious event detected in our study group.

Table 2
Reported adverse events in this study (n = 39)

| Adverse events     | Number |
|--------------------|--------|
| Skin itchiness     | 1      |
| Anorexia           | 5      |
| Diarrhea           | 1      |
| Constipation       | 2      |

Discussion

The possible causes for eradication therapy failure include antibiotic resistance, smoking, bacterial load before treatment, bacterial genotype, poor patient compliance, and polymorphisms of metabolism of PPIs. With the increasing prevalence of antimicrobial resistance, the eradication rate of *H. pylori* have been declined. The Maastricht IV/Florence consensus report recommends that culture and antimicrobial sensitivity testing should be performed after one or two treatment failures with different antibiotics [3]. Meanwhile, according to the Maastricht V/Florence consensus report, after the first failure, if endoscopy is carried out, culture and standard antimicrobial susceptibility testing are recommended to tailor the treatment [11].

The prevalence of *H. pylori* strains resistant to more than one antibiotic was 15% in the United States and 8.9% in Europe [12]. According to the study of Liou et al. [13], the secondary resistance rates of
clarithromycin, levofloxacin, and metronidazole were as high as 92.5%, 70.1%, and 87.7%, respectively, in patients who had received these antibiotics in their prior therapies in Taiwan.

When selecting salvage therapy, previously used antibiotics should be avoided. The use of a salvage regimen for patients with persistent \( H. pylori \) infection is an increasingly common scenario but remains a challenge for clinicians because only a few antibiotics are available. Currently, a standard salvage regimen is still lacking. Our data showed 10 days rifabutin-based triple therapy was well tolerated and yielded an acceptable \( H. pylori \) eradication rate for patients infected with dual drug-resistant strains to clarithromycin and levofloxacin.

Rifabutin inhibits the beta-subunit of DNA-dependent RNA polymerase of \( H. pylori \), which is encoded by the \( rpoB \) gene. Rifabutin-based triple therapy has been applied as a rescue treatment. A low rate of resistance (0.24%) to rifabutin was noted in \( H. pylori \) strains isolated from 414 Japanese patients. The only rifabutin-resistant strain detected showed a point mutation in the \( rpoB \) gene and was isolated from a patient with a history of rifampin treatment for pulmonary tuberculosis. The mean \( H. pylori \) rifabutin resistance rate (calculated from 11 studies, including 2982 patients) was 1.3% (95% confidence interval [CI], 0.9–1.7%) [14]. The respective cure rates for second-line (223 patients), third-line (342 patients), and fourth/fifth-line (95 patients) rifabutin therapies were 79% (95% CI, 67–92%), 66% (95% CI, 55–77%), and 70% (95% CI, 60–79%), respectively [14].

The American College of Gastroenterology clinical guideline suggests a rifabutin triple regimen consisting of a PPI, amoxicillin, and rifabutin for 10 days as a suggested salvage regimen, but it has a very low quality of evidence for duration [15]. The ideal length of treatment for the rifabutin regimen remains unclear. In some reports, a 7-day course has been equally effective as the 10- to 14-day regimens, whereas others have found that this shorter duration dramatically reduced the efficacy in terms of eradication rates. High-dose proton pump inhibitor seems to play some role. One Korea study demonstrated that higher eradication rate was achieved when double doses (lansoprazole 60 mg bid) were administered instead of standard doses (lansoprazole 30 mg bid) with the same rifabutin-amoxicillin combination (intention-to-treat, 96.3% vs 78.1%. \( p = 0.51 \)) [19].

A recent study by Fiorini et al. [16] reported that the efficacy of the 12-day rifabutin-based triple therapy (with esomeprazole 40 mg bid, amoxicillin 1 g bid, and rifabutin 150 mg od) for patients infected with multidrug-resistant strains (clarithromycin, metronidazole, and levofloxacin) was 82.9% (95% CI, 78.3–87.5) by intention-to-treat analysis and 88.7% (95% CI, 84.7–92.7) at per-protocol analysis. The mean rate of adverse effects was 22% (19–25%). A long-term prospective study in a large cohort with 302 difficult-to-treat patients revealed that rifabutin 150 mg, amoxicillin 1 g and a standard dose of proton pump inhibitor, twice daily for 14 days achieved eradication rate in 72.7% (per-protocol) and 71.5% (intention-to-treat) respectively. A univariate analysis showed that gender, ethnic background, smoking habits and familial history of gastric diseases were not predictive factors of response [17]. The efficacy of rifabutin treatment is summarized in Table 4.
| Author(s) and year | Country | Drugs and doses | Duration of treatment (days) | No. of patients | No. of previously failed treatment | Eradication rate (%) |
|--------------------|---------|----------------|-----------------------------|----------------|-----------------------------------|---------------------|
| Fiorini G 2018 [16] | Italy   | esomeprazole 40 mg bid, amoxicillin 1 g bid, and rifabutin 150 mg od | 12              | 254            | 2                                | 82.9% intention-to-treat |
| Ribaldone DG 2019 [17] | Italy   | Rifabutin 150 mg bid, Amoxicillin 1 g bid, PPI bid | 14              | 302            | 2                                | 71.5% intention-to-treat |
| Van Zanten et al. 2010 [18] | Canada | Rifabutin 300 mg od, Amoxicillin 1 g bid, PPI bid | 7               | 16             | 3                                | 63%                 |
| Lim et al. 2014 [19] | Korea   | Rifabutin 150 mg bid, Amoxicillin 1 g tid, Lansoprazole 60 mg bid | 7               | 27             | 2                                | 96.3% intention-to-treat |
| Lim et al. 2014 [19] | Korea   | Rifabutin 150 mg bid, Amoxicillin 1 g tid, Lansoprazole 30 mg bid | 7               | 32             | 2                                | 78.1% intention-to-treat |
| Author(s) and year | Country | Drugs and doses                                                                 | Duration of treatment (days) | No. of patients | No. of previously failed treatment | Eradication rate (%) |
|--------------------|---------|---------------------------------------------------------------------------------|-----------------------------|-----------------|----------------------------------|---------------------|
| Ierardi et al. 2014 [20] | Italy   | Rifabutin 150 mg bid                                                           | 10                          | 21              | 2                                | 77.7%               |
|                    |         | Minocycline 100 mg bid                                                        |                             |                 |                                  |                     |
|                    |         | Bismuth 120 mg qid                                                            |                             |                 |                                  |                     |
|                    |         | Rabeprazol 20 mg bid                                                          |                             |                 |                                  |                     |

One significant concerning in rifabutin treating was adverse effects of myelotoxicity. Lower doses and/or a shorter duration would lower the possibility of myelotoxicity. In the present study, we used rifabutin 150 mg bid, amoxicillin 1 g bid and esomeprazole 40 mg bid for 10 days and no case of neutropenia was observe.

**Conclusions**

Our current study demonstrated that 10 days rifabutin-based triple therapy was well tolerated and yielded an acceptable eradication rate for patients infected with dual drug-resistant *H. pylori*.

**Abbreviations**

*H. pylori: Helicobacter pylori, PPI: proton pump inhibitor*

**Declarations**

**Authors’ contributions** Study design and idea: Lin CJ, Chiu CT, Su MY; Data acquisition: Lin CY, Le PH, Cheng HT. Analysis of data: Hsu JT, Tseng CN, Chang ML. Writing of manuscript: Kuo CJ, Lin WR. Revision of manuscript: Chang PY, Lai CH, Hsieh SY. All authors have read and approved the manuscript in the current state.

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**Availability of data and materials** The datasets used and analysed during the current study are available from the corresponding author on reasonable request.
Ethics approval and consent to participate This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (IRB No. 201701000A3). Written informed consent was obtained from all patients.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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Figures
Figure 1

Schematic flow chart