Empirical modified sequential therapy containing levofloxacin and high-dose esomeprazole in second-line therapy for Helicobacter pylori infection: a multicentre clinical trial

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Objectives: Sequential therapy appears to achieve a higher Helicobacter pylori eradication rate than triple therapy. We assessed the efficacy and tolerability of modified sequential therapy containing levofloxacin and high-dose esomeprazole in second-line therapy.

Methods: Patients who failed first-line triple therapy with clarithromycin, amoxicillin and a proton pump inhibitor were eligible in this multicentre trial. Eligible patients were treated with esomeprazole 40 mg and amoxicillin 1 g for the first 5 days, followed by esomeprazole 40 mg, levofloxacin 250 mg and metronidazole 500 mg for another 5 days (all given twice daily). Eradication was confirmed with a 13C-urea breath test 6 weeks after therapy. Drug susceptibility, presence/absence of gyrA mutation and CYP2C19 genotype were also determined.

Results: A total of 142 patients were enrolled. The eradication rate was 95.1% [135/142, 95% confidence interval (CI) 91.5%–98.6%] in the intention-to-treat analysis and 96.4% (133/138, 95% CI 93.3%–99.5%) in the per protocol analysis. Four patients (2.8%) failed to take at least 80% of the drugs due to adverse effects. The eradication rates were 50% (4/8) and 97.7% (43/44) in patients with and without metronidazole resistance, respectively (P=0.001). The eradication rates were 84.6% (11/13) and 95.1% (58/61) in patients with and without gyrA mutation, respectively (P=0.210). The eradication rates were not affected by the CYP2C19 polymorphism (P=0.421).

Conclusions: This modified sequential therapy achieved an excellent eradication rate (>95%) in second-line treatment and the eradication rate appeared to be affected by metronidazole resistance.

Keywords: metronidazole, gyrA, CYP2C19, resistance, rescue

Introduction

Helicobacter pylori plays a crucial role in the pathogenesis of peptic ulcer diseases and gastric cancer.1–3 Eradication of H. pylori has been shown to reduce the risk of recurrence of peptic ulcer diseases and even the development of gastric cancer.4,5 Standard triple therapy containing clarithromycin, amoxicillin and a proton pump inhibitor (PPI) for 7–14 days is still the recommended first-line therapy according to the US and European guidelines.5,6 However, many studies have shown that the eradication rate of the standard triple therapy has fallen below 80% in many countries.7 Therefore, several alternative first-line regimens have been proposed in order to reach an eradication rate of 90% or greater.7–14 Sequential therapy consisting of a PPI plus amoxicillin for 5 days, followed by a PPI plus clarithromycin and tinidazole (or metronidazole) for another 5 days, has been shown to be more effective than the standard triple therapy in first-line treatment in Italy.8–12 More importantly, the eradication rate of sequential therapy was not significantly reduced in the presence of clarithromycin.

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resistance.8–12 Therefore, the sequential regimen would be a promising alternative first-line therapy if the good eradication rate could be validated in other countries.

Another strategy to overcome the declining eradication rate in first-line therapy is to develop an effective second-line rescue regimen. The rationale for this strategy is that the cumulative eradication rate could be as high as 98% if the first-line and second-line eradication rates were 80% and 90%, respectively. Recently, levofloxacin-based triple therapy was shown to be more effective and better tolerated than quadruple therapy in second- and third-line treatment for H. pylori infection.15–17 However, neither quadruple therapy nor levofloxacin-based triple therapy achieved a grade A or B successful eradication rate (≥90%) in second- and third-line treatment.15–18 Recent studies showed that a higher dose of PPI was associated with a higher eradication rate because the efficacies of amoxicillin and clarithromycin were affected by pH values.19,20 Because the MIC of levofloxacin was also affected by the pH value,21 we hypothesized that the use of a higher dose of PPI might lead to a higher eradication rate for levofloxacin-based therapy. Besides, as it has been reported that the combination of ofloxacin and metronidazole shows a synergistic effect against anaerobic bacteria,22,23 we hypothesized that the addition of metronidazole might also lead to a higher eradication rate for levofloxacin-based therapy in the treatment of H. pylori infection. Therefore, we assessed the efficacy and tolerability of levofloxacin-based sequential therapy in the second-line treatment of H. pylori infection. We also assessed the impacts of levofloxacin and metronidazole resistance and the CYP2C19 polymorphism on eradication rates.

Materials and methods

This prospective, open-label, multicentre trial was conducted at the National Taiwan University Hospital, Yun Lin Branch of National Taiwan University Hospital, and E-DA Hospital from April 2009 to December 2010. The study protocol was approved by the Institutional Review Boards of National Taiwan University Hospital and E-DA Hospital. Written informed consent was obtained from all patients prior to enrolment. The ClinicalTrials.gov registration identifier was NCT00885417.

Eligibility criteria

H. pylori-infected patients who failed first-line standard triple therapy with clarithromycin, amoxicillin and a PPI were eligible for this study. Patients were excluded from the study if any one of the following criteria was present: (i) children and teenagers aged <20 years; (ii) history of gastrectomy; (iii) gastric malignancy, including adenocarcinoma and lymphoma; (iv) previous allergic reaction to antibiotics (amoxicillin, metronidazole, levofloxacin) and PPI (esomeprazole); (v) contraindication to treatment drugs; (vi) pregnant or lactating women; or (vii) severe concurrent disease.

Determination of H. pylori status

Prior to enrolment, the status of H. pylori infection was determined by either the 13C-urea breath test (13C-UBT) or the rapid urease (CLO) test, histology and culture. Patients with either one positive 13C-UBT or any two positive of CLO, histology and culture were defined as refractory to previous treatment and were eligible for enrolment. Oesophago-gastro-duodenoscopy (OGD) was performed for patients enrolled with the second criterion. During OGD, biopsies from the antrum (one piece for the CLO test, two pieces for culture, and two pieces for histology) and body (two pieces for histology) were taken from these patients. Patients enrolled with the first criterion were also invited to undergo OGD examination and biopsy, but this was not compulsory. After second-line treatment, H. pylori status was determined by 13C-UBT at least 6 weeks after the completion of eradication therapy. Successful eradication of H. pylori was defined as a negative 13C-UBT result. The assessment of 13C-UBT was centralized at the Taipei Institute of Pathology, Taipei, Taiwan. A positive 13C-UBT test was defined as a 8 value of 4 units or greater. All subjects were asked to stop PPI and H2-blockers for at least 2 weeks before 13C-UBT.

Therapy and assessment of adverse effects

Enrolled patients were treated with esomeprazole 40 mg and amoxicillin 1 g for the first 5 days, followed by esomeprazole 40 mg, levofloxacin 250 mg and metronidazole 500 mg for another 5 days (all given twice daily). At enrolment, the patients were informed of the common side effects of the study drugs and were asked to record these symptoms during treatment. A standardized interview and questionnaire were used to assess the adverse events and compliance at the end of treatment. Patients with low compliance, as defined by taking <80% of the pills, and those lost to follow-up were excluded from the per protocol analysis.

Susceptibility test and genotyping of gyrA

The biopsy specimens were cultured on plates containing Brucella chocolate agar with 7% sheep blood and incubated for 7 days under microaerobic conditions. H. pylori DNA was extracted from homogeneous bacterial strains isolated by culture using a DNA extraction kit (Gentra DNA Purification Kit, Qiagen, USA) according to the manufacturer’s instructions. All culture isolates and DNA samples were stored at −80°C until use. The MIC was determined by the agar dilution test. The resistance breakpoints for amoxicillin, clarithromycin, levofloxacin and metronidazole were defined as ≥0.5, ≥1, ≥1 and ≥8 mg/L, respectively. The genotypes of gyrA and 23S rRNA were determined by PCR followed by direct sequencing using an automatic sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems), as described previously.24 The primers for the Hpa23S fragment were as follows: forward, 5′-CCACAGGCATGTGTCTCGAG-3′; reverse, 5′-TCTCTATAAGGCCAAGCCAAGCC-3′. The primers for the gyrA gene were as follows: forward, 5′-TTRGCTATTCTCATTAGGCTT; reverse, 5′-GCCAGGGCTTGGTARAAAT. The technicians who performed the susceptibility test and genotyping were blind to each other’s results and the therapeutic outcomes.

Genotyping of CYP2C19

Genomic DNA from peripheral blood lymphocytes was used to assess the CYP2C19 wild-type allele and the two known mutant alleles, CYP2C19*1/*1 in exon 5 and CYP2C19*2/*2 in exon 4. PCR–restriction fragment length polymorphism (PCR–RFPL) techniques with allele-specific primers as previously reported were used for genotyping.25 Patients were classified into the following three genotype groups: rapid metabolizer (RM, *1/*1), intermediate metabolizer (IM, *1/*2 or *1/*3) and poor metabolizer (PM, *2/*2, *3/*3 or *2/*3) as reported previously.25

Sample size estimation and statistical analysis

In our pilot study, the point estimate of the eradication rate was about 97%. Therefore, we assumed the estimated efficacy to be 97%, ε (the desired confidence interval) to be 3% and the desired confidence level to be 95%. The minimum sample size was 124 based on these
assumptions. Therefore, the final size of this study should be at least 136 patients assuming 10% are lost to follow-up. The primary endpoint of the study was the eradication rate. The secondary endpoints were the tolerability and adverse effects of the regimen. Categorical data were compared using the χ² test or Fisher’s exact test as appropriate. Continuous data were compared with Student’s t-test and expressed as mean (SD). The κ coefficient was used to assess the agreement between genotypic resistance and phenotypic resistance. All P values were two-tailed, with the level of statistical significance specified as 0.05. The statistical analyses were performed using SPSS 12.0 statistical software for Windows.

Results

Demographic data and prevalence of antibiotic resistance

Participant flow in this study is shown in Figure 1. Gastric biopsy specimens were available for genotypic resistance determination in 80 (56.3%) patients (Table 1). H. pylori strains were available for the agar dilution test in 52 (36.6%) patients. Genotypic resistance was determined from the H. pylori strains if available or gastric biopsy specimens if strains were not available. The successful genotyping rates were 98.8% (79/80) and 92.5% (74/80) for 23S rRNA and gyrA, respectively. The prevalence of clarithromycin genotypic (23S rRNA mutation) resistance and phenotypic resistance from the strains were 51.9% (41/79) and 63.5% (33/52), respectively (Table 1). The prevalence of levofloxacin genotypic (gyrA mutation) and phenotypic resistance were 17.6% (13/74) and 15.4% (8/52). The phenotypic and genotypic resistance from the strains correlated well for both clarithromycin (κ = 0.751) and levofloxacin (κ = 0.866). The prevalences of amoxicillin resistance and metronidazole resistance were 1.9% (1/52) and 15.4% (8/52), respectively.

Eradication rates

The eradication rate was 95.1% [135/142, 95% confidence interval (CI) 91.5%–98.6%] in the intention-to-treat analysis and 96.4% [133/138, 95% CI 93.3%–99.5%] in the per protocol analysis (Table 2). Among the four patients excluded from the per protocol analysis, eradication was successful in two patients and failed in two patients. The eradication rates were not significantly different with respect to the CYP2C19 polymorphism (P = 0.421) and levofloxacin resistance, as shown in Table 3. The eradication rates were 50% (4/8, 95% CI 15.4%–84.7%) and 97.7% (43/44, 95% CI 93.3%–100%) in patients with and without metronidazole resistance, respectively (P = 0.001 by Fisher’s exact test, Table 3). When the strains were susceptible to metronidazole, the eradication rates were 97.4% and 100% for strains susceptible and resistant to levofloxacin, respectively. However, when the strains were resistant to metronidazole, the eradication rates were 66.7% and 0% for strains susceptible and resistant to levofloxacin, respectively. The eradication rates were 97.6% (81/83) and 91.5% (54/59) in patients with and without peptic ulcer disease, respectively (P = 0.127).

Adverse effects and compliance

Adverse effects were reported in 65 (45.8%) patients, the main adverse effects being dizziness (19.0%), nausea (15.5%), diarrhoea (12.7%) and taste distortion (11.3%), as shown in Table 4. Most (86.2%, 56/65) of these adverse effects were mild. Seven patients (4.9%) discontinued the drugs due to adverse effects and four (2.8%) failed to take at least 80% of the study drugs (one of them was also lost to follow-up) (Figure 1). In total, 96.5% (137/142) of patients took the medications correctly (Table 4).

Discussion

This is the first study to show the excellent eradication rate (>95%) and good tolerability of this modified sequential therapy in second-line treatment for H. pylori infection. We also report the novel finding that the eradication rate of this regimen appeared to be affected by metronidazole resistance, but not by levofloxacin resistance. Interestingly, the eradication rate was 0% in the presence of dual levofloxacin and metronidazole phenotypic resistance. If historical controls from our previous randomized trial were used, we found that the modified sequential therapy containing high-dose esomeprazole appeared to achieve a higher eradication rate (95.1%, 135/142)
than did the levofloxacin-based triple therapy containing standard-dose lansoprazole (80%, 20/25, P = 0.019) in second-line treatment. However, further randomized control trials (RCTs) with larger sample size are still warranted to confirm the superiority of this modified sequential regimen over the triple therapy regimen.

There are two major factors that might have contributed to the high eradication rate. Firstly, we used the sequential regimen containing metronidazole in addition to amoxicillin and levofloxacin. Secondly, we used a higher dose of esomeprazole rather than standard-dose PPI. A previous pharmacodynamic study showed a synergistic effect of clarithromycin and tinidazole against *H. pylori*. The combination of ofloxacin and metronidazole was also shown to exert synergistic effects against anaerobic bacteria. The results of our study suggested a possible synergistic effect of metronidazole and levofloxacin against *H. pylori*. However, further in vitro studies are warranted to confirm this synergistic effect.

In the clarithromycin-based sequential therapy, the eradication rate was not affected by the presence of clarithromycin resistance. Our study showed that the eradication rate of the modified sequential therapy was affected by metronidazole resistance, but was not affected by levofloxacin resistance. Our result was contradictory to a recent study which showed that the eradication rate of the modified sequential therapy containing levofloxacin was not affected by metronidazole resistance. The discrepancy between these two studies was probably related to the different nitroimidazole used and the different tests used for MIC determination. In this study, we used metronidazole rather than tinidazole. A previous study showed that the use of tinidazole in combination with clarithromycin appeared to give a better synergistic effect than did the combination of metronidazole and clarithromycin. Secondly, we used the agar dilution test in this study, whereas Romano et al. used the Etest. Previous studies have shown a lack of inter- and intra-laboratory reproducibility in the determination of the MIC of metronidazole against *H. pylori* in most studies. Future studies with larger sample sizes are warranted to address this issue.

The efficacy and tolerability of the modified sequential therapy containing levofloxacin in the treatment of *H. pylori* infection has been reported in some recent studies with different results. Romano et al. showed that modified sequential therapy containing levofloxacin achieved higher eradication (95%) than clarithromycin-based sequential therapy (80%) in first-line therapy in Italy. Molina-Infante et al. reported that the eradication rate of modified sequential therapy containing levofloxacin was 82.5% in first-line therapy. However, the eradication rates of modified sequential therapy containing levofloxacin were only about 75.7% (28/37) and 77.8% (21/27) in second-line therapy in Turkey and Greece, respectively. As our result showed that the eradication rate of the modified sequential therapy was affected by metronidazole resistance, it is not surprising that the eradication rates of this modified therapy were lower in second-line therapy.
regimen were lower in Turkey and Greece, where the prevalences of metronidazole resistance were higher.29,30

Previous studies showed that the MICs of amoxicillin and clarithromycin were affected by pH values, with higher MIC and shorter half-life for antibiotics in a more acidic environment.31

A recent meta-analysis also showed that the use of a higher PPI dose was associated with an 8% increase in the eradication rate compared with the standard dose of PPI.19 Therefore, we decided to use a higher dose of esomeprazole twice daily in the modified sequential regimen in an attempt to achieve a good eradication rate (>90%). The efficacy of triple therapy containing levofloxacin was shown to be affected by the CYP2C19 polymorphism.32 However, our study indicated that the use of a higher dose of esomeprazole could probably overcome the influence of CYP2C19 polymorphisms on the eradication rate of the modified sequential therapy.

The Maastricht III Consensus Report recommends that clarithromycin-based triple therapy should not be used as empirical first-line treatment in areas with resistance above 15%–20%.5 In Taiwan, the primary clarithromycin resistance rate for H. pylori ranged from 9.3% to 13.5%.26,33 Therefore, clarithromycin-based triple therapy could still be used empirically in first-line therapy. The secondary prevalence of clarithromycin resistance was as high as 52% in this study. This result was consistent with a previous study that showed a higher prevalence (46%) of clarithromycin resistance strains in patients who failed first-line therapy compared with treatment-naive patients (18%).34 It is noteworthy that mixed susceptible and resistant strains had been reported in the same stomach in about 10%–25% of patients.35 Therefore, caution should be exercised when single clones isolated from culture are used for the analysis of antibiotic resistance in future studies.

There were some limitations of this study. Firstly, this was a single-arm uncontrolled study. Therefore, it is still unknown whether the modified sequential therapy is better than the levofloxacin-based triple therapy in second-line therapy. The reason why we chose to conduct this single-arm study was that our objective was to identify a highly effective (>90%) and well tolerated rescue regimen that deserves further RCTs. Besides, the case number required in RCTs to identify a 13% difference (93% versus 80%, α=0.05, power=0.8) should be at least 240, which would take a longer time for case enrolment in the second-line treatment. Secondly, levofloxacin genotypic and phenotypic resistances were available in only 52.1% (74/142) and 36.6% (52/142) of patients, respectively. Because many of these patients had received an OGD several months before enrolment in this study, only 56.3% (80/142) of them agreed to receive another endoscopy for H. pylori culture before second-line therapy. Thirdly, the prevalence of metronidazole resistance was only about 15% in the present study. Because the eradication rate appeared to be compromised in the presence of metronidazole resistance, similar results would not be expected in regions with high levels of metronidazole resistance, such as in Turkey and Greece. Finally, the rate of adverse effects was high (45.8%) and 4.9% patients discontinued the drugs due to adverse effects in this study. The higher rate of adverse effects was probably attributable to the active surveillance of the 10 most common subjective adverse effects, as identified using a questionnaire. We considered that the actual rate of adverse effects might have been lower because these symptoms were subjective and a placebo-controlled group was lacking in the present study.

In conclusion, the modified sequential therapy is an attractive second-line regimen with a high eradication rate and good tolerability, and deserves further validation in RCTs in other parts of the world. The impacts of CYP2C19 polymorphism and higher doses of PPI on the eradication rates of clarithromycin-based sequential therapy also deserve further investigation.

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Transparency declarations

None to declare.

Author contributions

Each author contributed sufficiently to the work to take public responsibility for the content of the paper and approved the final version of the manuscript. Jyh-Ming Liou and Chieh-Chang Chen contributed equally to this work. Concept and design: Jyh-Ming Liou, Chieh-Chang Chen, Jaw-Town Lin and Ming-Shiang Wu. Acquisition of data: all authors. Analysis and interpretation of data: Jyh-Ming Liou, Chieh-Chang Chen, Jaw-Town Lin and Ming-Shiang Wu. Drafting of the manuscript: Jyh-Ming Liou and Chieh-Chang Chen. Critical revision of the manuscript for important intellectual content: Jaw-Town Lin and Ming-Shiang Wu. Statistical analysis: Jyh-Ming Liou. Obtained funding: Jyh-Ming Liou, Jaw-Town Lin and Ming-Shiang Wu. Administrative, technical and material support: Jaw-Town Lin and Ming-Shiang Wu. Study supervision: Jaw-Town Lin and Ming-Shiang Wu.

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