VGHKS104-062

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Research Project Title：A prospective randomized trial of levofloxacin-amoxicillin triple therapy vs. levofloxacin-tetracycline quadruple therapy in second-line *Helicobacter pylori* treatment

NCT Number：NCT02978157

Date of the document：2017/09/30

ABSTRACT

Background: Although proton pump inhibitor（PPI）-amoxicillin-levofloxacin triple therapy has been recommended by the Maastricht IV/Florence Consensus Report for recue treatment of both standard triple therapy and non-bismuth quadruple therapy, the eradication rates of the rescue treatment for standard triple therapy and non-bismuth quadruple therapy by intention-to-treat analysis are only 66.9% and 77.5%, respectively. It is therefore mandatory and urgent to develop a highly effective rescue therapy for second-line treatment of *H pylori* infection. From the profiles of antibiotic susceptibility data following eradication therapy, tetracycline, amoxicillin and levofloxacin are all good candidates of antibiotics used in the rescue treatment for both standard triple therapy and non-bismuth quadruple therapy. Our recent study also revealed that PPI- bismuth-tetracycline-levofloxacin rescue treatment might achieve a very high eradication rate 96% for *H pylori* infection after failure of non-bismuth quadruple therapy.

Aims: To investigate (1) the efficacy of PPI- bismuth -levofloxain-tetracycline quadruple therapy and PPI-amoxicillin-levofloxacin triple therapy in rescue treatment for *H pylori* infection, and (2) the bacterial and host factors determining the efficacy of PPI-bismuth-levofloxain-tetracycline quadruple therapy and PPI-amoxicillin-levofloxacin triple therapy.

Methods: Consecutive 164 *H pylori*-infected adult patients with failure of first-line therapies are randomly assigned to either EBLT (esomeprazole 40 mg b.d., bismuth 120 mg q.d.s., levofloxacin 500 mg o.d., and tetracycline 500 mg q.d.s.) therapy or EAL (esomeprazole 40 mg b.d., amoxicillin

500 mg q.d.s., and levofloxacin 500 mg o.d.) for 10 days. Additionally, antibiotic susceptibility of *H pylori* and *CYP2C19* polymorphism of the hosts are examined. Urea breath test is performed at six weeks after the end of anti-*H pylori* therapy. The rates of eradication, adverse events and compliance will be compared between EAL and EBTL group by chi-square test. Besides, the host and bacterial factors influencing the efficacy of above two salvage regimens are assessed.

INTRODUCTION

Helicobacter pylori (*H pylori*) infect more than 50% of humans globally. It is the principal cause of chronic gastritis, gastric ulcer, duodenal ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma (MALToma) [1,2]. *H pylori* eradication has become the standard and most widely adopted therapy to cure peptic ulcer disease [3]. This therapy is also strongly recommended in the treatment of *H pylori*-related MALToma [4]. In regions with high incidence of gastric adenocarcinoma, eradication of *H pylori* is advocated as a preventative measure [5].

Standard triple therapy has been recommended as first-line regimen for *H pylori* infection in main international guidelines [6,7]. However, several large clinical trials and meta-analyses have shown that the eradication rate of the standard therapy has generally declined to unacceptable levels (i.e., 80% or less) recently [8,9]. In some European countries, the success rates are disappointingly low with values only 25-60% [10,11]. Therefore, several novel first-line therapies including sequential therapy, concomitant therapy and hybrid therapy have emerged to treat naive *H pylori* infection [12-14].

The Maastricht IV/Florence Consensus Report [15] has recommended treatment of *H pylori* infection according to antibiotic resistance rates in local areas recently. In some countries with low clarithromycin resistance (< 15%) of *H pylori*, standard triple therapy is still the best option for first-line anti-*H pylori* therapy, but bismuth-containing quadruple therapies such as sequential therapy and concomitant therapy are the preferred option in countries with clarithromycin resistance >20%.

In clinical practice, the regimen for second-line therapy of *H pylori* infection depends on the regimen used in first-line therapy. After failure of a standard triple therapy, either a bismuth-containing quadruple therapy or PPI-amoxcillin-levofloxacin triple therapy is recommended [15]. Bismuth–containing quadruple therapy regimen fails in 5-63% of patients with an average eradication rate of 76% on the basis of a pooled analysis [16-18]. PPI-amoxcillin-levofloxacin has been recommended as an encouraging strategy for second-line therapy [19]. A meta-analysis by Saad et al. [19] showed that a 10-day regimen of levofloxacin-based triple therapy was superior to 7-day bismuth-based quadruple therapy. However, levofloxin-based triple therapies seem less effective in Asia. Our recent study showed that PPI-amoxicillin-levofloxacin triple therapy only achieved a 69.9% eradication rate for rescue treatment of standard triple therapy in Taiwan [20].

Currently, the best second-line therapy after failure of non-bismuth quadruple therapies (such as sequential therapy, concomitant therapy or hybrid therapy) remains unclear. Although PPI-amoxicillin-levofloxacin triple therapy also has been recommended by the Maastricht IV/Florence Consensus Report as a rescue treatment for sequential therapy [15], the mean eradication rate of the rescue regimen is suboptimal (mean, 77.5% [79/102]; range: 50% [3/6] to 100% [7/7]) [21-25].

Antibiotic resistance is the key factor determining the outcome of rescue treatment for anti-*H pylori* therapy. According to our previous reports [20,26], the drug resistant rates to tetracycline, levofloxacin, amoxicillin, clarithromycin and metronidazole were 0%, 21%, 6%, 59% and 57% respectively following standard triple therapy [20] and 0%, 25%, 0%, 75% and 75% respectively following sequential therapy [26]. The data indicate that tetracycline, amoxicillin and levofloxacin are good candidates of antibiotics used in the rescue treatment for both standard triple therapy and non-bismuth quadruple therapy (such as sequential therapy, concomitant therapy and hybrid therapy).

Although PPI-amoxicillin-levofloxacin triple therapy has been recommended by the Maastricht IV/Florence Consensus Report for recue treatment of both standard triple therapy

and non-bismuth quadruple therapy [15], the eradication rates of the rescue treatment for standard triple therapy and sequential therapy by intention-to-treat analysis are only 66.9% [20] and 77.5% [23], respectively. Our recent study also revealed that PPI- bismuth-tetracycline-levofloxacin rescue treatment might achieve a very high eradication rate 96%) for *H pylori* infection after failure of non-bismuth quadruple therapy [26]. We therefore design the prospective, multicenter, randomized controlled trial to investigate (1) the efficacy of PPI-bismuth -levofloxain-tetracycline quadruple therapy and PPI-amoxicillin-levofloxacin triple therapy in rescue treatment for standard triple therapy, (2) the efficacy of PPI-bismuth -levofloxain-tetracycline quadruple therapy and PPI-amoxicillin-levofloxacin triple therapy in rescue treatment for nonbismuth-containing quadruple therapy (such as sequential therapy, concomitant therapy and hybrid therapy), and (3) the bacterial and host factors determining the efficacy of PPI- bismuth- levofloxain-tetracycline quadruple therapy and PPI-amoxicillin-levofloxacin triple therapy.

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PATIENTS AND METHODS

Patients

Consecutive 164 *H pylori*-infected adult patients (≥ 20 years old) with failure of first-line therapy for *H pylori* infection will be enrolled for this study after giving informed consent. The presence of *H pylori* after a previous eradication therapy is defined as (1) positive results of both rapid urease test and histology, (2) a positive result of ^{13}C urea breath test, or (3) a positive result of culture. Criteria for exclusion included (a) ingestion of antibiotics, bismuth, or PPI within the prior 4 weeks, (b) patients with allergic history to the medications used, (c) patients with previous gastric surgery, (d) the coexistence of serious concomitant illness (for example, decompensated liver cirrhosis, uremia), and (e) pregnant women.

Methods

Using a computer-generated number sequence, the eligible patients are randomized to either EBTL (esomeprazole 40 mg b.d., bismuth 120 mg q.d.s., levofloxacin 500 mg o.d., and

tetracycline 500 mg q.d.s.) therapy or EAL (esomeprazole 40 mg b.d., amoxicillin 500 mg q.d.s., and levofloxacin 500 mg o.d.) for 10 days. All drugs are taken one hour before meals or night sleep.

Patients are asked to return at the 2nd week to assess drug compliance and adverse events. To assess eradication efficacy, ^{13}C urea breath tests are conducted to assess *H. pylori* status.

Demographic data

A complete medical history and demographic data are obtained from each patient, including age, sex, medical history, history of smoking, alcohol, coffee and tea consumption. Smoking was defined as consumption of cigarettes 1 pack or more per week. Coffee or tea consumption is defined as drinking 1 cup or more per day.

Adverse events are prospectively evaluated. The adverse events are assessed according to a 4-point scale system: none; mild (discomfort annoying but not interfering with daily life); moderate (discomfort sufficient to interfere with daily life); and severe (discomfort resulting in discontinuation of eradication therapy) [26,27]. Compliance is checked by counting unused medication at the completion of treatment. Poor compliance is defined as taking less than 80% of the total medication.

Histological examination

Two biopsy specimens are taken from the lesser curvature sites of the antrum and the corpus, respectively. They are fixed in 10% buffered formalin, embedded in paraffin, and sectioned. The sections, 4-μm thick, are stained with a haematoxylin and eosion stain and a modified Giemsa stain to observe the presence of curved rod shape bacteria on the mucosal surface [28]. Biopsy specimens are assessed by a histopathologist (HH Tseng), blinded to patient status and the results of other laboratory tests.

Urea breath test

The urea breath test is performed according to our previous studies [29,30]. The staffs who are

blind to the *H pylori* status perform the tests.

Culture and antimicrobial resistance

One antral gastric biopsy specimen is obtained for isolation of *H pylori,* using previously described culture methods [14]. All stock cultures are maintained at –80°C in Brucella broth (Difco. Detroit, MI) supplemented with 20% glycerol (Sigma Chem. Co. St. Louis, MO). The organisms are identified as *H pylori* by Gram staining, colony morphology, and positive oxidase, catalase, and urease reactions. As previously described in more detail [8], the antibiotic susceptibility is tested by E test (AB Biodisck, Solna, Sweden). *H pylori* strains with a MIC value > 0.5 μg/mL, > 1 μg/mL, > 4 μg/mL, > 8 μg/mL and > 1 μg/mL are considered to be resistant to amoxicillin, levofloxacin, tetracycline, metronidazole and clarithromycin, respectively [14,16].

Genotyping of *CYP2C19*

Blood sampling for genotyping of CYP2C19 is carried out before endoscopy for the subjects who provide informed consent for genetic study. The *CYP2C19* genotype is determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [31]. A two single-tube tetra-primer PCR assays is applied to detect the *CYP2C19*2* and the **3* allele, respectively. To genotype the *CYP2C19*2* polymorphism, four primers (Table 1) are combined in one tetra-primer PCR assay. Amplification of *CYP2C19* exon 5 with primers Ex5U and Ex5L produced a 321-bp product that served as internal control for the quality of the PCR amplification and as template for the allele-specific amplification (ASA). ASA produced the 229-bp PCR product specific for the **2* allele and the 127-bp PCR product specific for the wild-type allele. The tetra-primer PCR produced amplification of the 309-bp *CYP2C19* exon 4 region (Ex4U and Ex4L), and ASA (*3mutU and *3wtL) produced the 110-bp PCR product for the **3* allele and the 228-bp PCR product for the wild-type allele. The following reaction mixture was prepared for the tetra-primer PCR assay to genotype *CYP2C19*2*: 16.4 mL of water, 2.5 mL of buffer 1 (1.5 mM MgCl2), 0.2 mL of Gold Taq (5 U/mL), 0.5 mL of dNTP mixture (10 mM), 0.4 mL of Ex5U (10 mM), 0.5 mL of Ex5L (10 mM), 0.75 mL of *2mutU (10 mM), 0.75 mL of *2wtL (10 mM), and 3.0 mL of genomic

DNA (;50 ng/mL). The following cycling conditions were used: 10 min at 94 °C; 44 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s; and a final extension of 7 min at 72 °C. Separation by 2% agarose gel electrophoresis and ethidium bromide staining allowed identification of the three PCR products and the interpretation of the *CYP2C19*2* genotype.

Table 1. Oligonucleotides used in this study.

Primer	5′ position[a]	Sequence[b]	3′ position[a]
Exon 5			
2C19Ex5U[c]	8	CAGAGCTTGGCATATTGTATC	28
2C19Ex5L[c]	328	GTAAACACACAACTAGTCAATG	307
2C19*2mutU	100	ATCATTGATTATTTCCC**A**[d]	117
2C19*2wtL	134	AATTTGTTATGGGTTCC**C**	117
Exon 4			
2C19Ex4U[e]	21	TATGAAGTGTTTTATATCTAATGTTTACTCA	51
2C19Ex4L[f]	329	ACTTCAGGGCTTGGTCAATATAGA	306
2C19*3mutU	220	GTAAGCACCCCCTG**A**	234
2C19*3wtL	248	GGCCTTACCTGGAT**C**	234

Genotypes are classified into three groups: homEM (*CYP2C19**1/*CYP2C19**1); hetEM (*CYP2C19**1/*CYP2C19**2 and *CYP2C19**1/*CYP2C19**3); PM (*CYP2C19**2/*CYP2C19**2, *CYP2C19**2/*CYP2C19**3, and *CYP2C19**3/*CYP2C19**3).

Statistical analysis

The primary outcome variable is eradication rate. The secondary outcome variables are the rate of adverse events and compliance. Chi-square test with or without Yates correction for continuity and Fisher's exact test are used when appropriate to compare the major outcomes between groups using the SPSS program (version 10.1, Chicago, Illinois, USA). A *P* value less than 0.05 is considered statistically significant. The sample size is estimated 164 (82 subjects per group) in order to detect a difference of 10% in the eradication rate between the EAL therapy (assumed eradication rate of 70% and the EBTL therapy (assumed eradication rate of 80%) for rescue treatment.

Eradication rates are evaluated by ITT and per-protocol (PP) analyses. ITT analysis includes all randomized patients who have taken at least one dose of study medication. Patients whose infection status is unknown following treatment are considered treatment failures for the purposes of ITT analysis. The PP analysis excludes the patients with unknown *H. pylori* status following therapy and those with major protocol violations.

To determine the independent factors affecting the treatment response, Host and bacterial

parameters are analyzed by univariate analysis. These variables include the following: age (<60 or ≥60 years), gender, history of smoking, history of alcohol consumption (<80 g/day or ≥80 g/day), ingestion of coffee (<1 cup/day or ≥1 cup/day), ingestion of tea ((<1 cup/day or ≥1 cup/day), coexistence of a systemic disease (yes or no), previous history of peptic ulcer disease, endoscopic appearance (ulcer or gastritis), CYP2C19 polymorphism, drug compliance (good or poor), and antibiotic susceptibility. Those variables found to be significant by univariate analysis are subsequently assessed by a stepwise logistic regression method to identify independent factors for eradication outcome.