

Improved *Helicobacter pylori* Eradication Rate of Standard Triple Therapy by Adding Bismuth and Probiotic Supplement in Thai Patients

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ABSTRACT

Objective: Standard triple therapy for *H. pylori* eradication is no longer effective in many countries including Thailand. This study was designed to evaluate the efficacy of adding bismuth and probiotic supplement to standard triple therapy for *H. pylori* eradication in Thai patients.

Method: This prospective single-center study was conducted in Thailand. *H. pylori* infected gastritis patients were randomized to 7- day or 14-day standard triple therapy plus bismuth, with or without probiotic supplement. The treatment regimen consisted of bismuth subsalicylate (524 mg) tablets twice daily, lansoprazole (30 mg) twice daily, amoxicillin 1 g twice daily and clarithromycin MR 1 g once daily. Probiotic regimens (one serve of drinking yogurt) were composed of *Bifidobacterium lactis* $\geq 10^9$ CFU/serve, *Lactobacillus acidophilus* $\geq 10^9$ CFU/serve and *Lactobacillus paracasei* $\geq 10^9$ CFU/serve. The placebo was a drinking yogurt without probiotic. *H. pylori* infection was defined as a positive culture or two positive tests (CLO[®] test and histology); CYP2C19 genotyping and antibiotic susceptibility tests were carried out. *H. pylori* eradication was evaluated by ¹³C-UBT 2 weeks or more after treatment.

Results: 100 subjects were enrolled (25 to each of the 7- day and the 14-day regimens with probiotic supplement or placebo). Antibiotic susceptibility testing showed 36.7% metronidazole resistance and 1.1% of clarithromycin resistance. CYP2C19 genotyping revealed 40.8% RM, 49% IM and 10.2% PM. The eradication rate with 7- day or 14-day regimen with probiotic supplement achieved the eradication rate of 100%. Interestingly, the side effects with bitter taste in the 7- day regimen with probiotic supplement was significantly less than in the 7- day regimen with placebo (40% vs. 64%; $p=0.04$).

Conclusion: 7-day standard triple therapy plus bismuth and probiotic supplement provided an excellent cure rate (100%) with fewer side effects. The efficacy of this regimen was very effective regardless of the metronidazole resistance and CYP2C19 genotype. Adding probiotic could help reduce the side effects of medications.

Key words : Bismuth, triple therapy, *Helicobacter pylori*, probiotic, Thailand

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is gram-negative bacteria etiologically associated with chronic gastritis, peptic ulcer disease, and gastric cancer⁽¹⁻³⁾. *H. pylori* has proven difficult to cure, and standard triple therapy is no longer recommended as an empiric choice in most countries^(4, 5). However, triple therapy is still recommended in areas where clarithromycin resistance is low, or when therapy is chosen based on pretreatment susceptibility testing. Triple therapy is adversely affected by many factors besides antibiotic resistance, including smoking, the doses utilized, and duration of therapy. For example, extending the duration of proton pump inhibitor (PPI)-clarithromycin-containing triple therapies from 7 to 10-14 days improves the eradication success by about 5%⁽⁶⁾. Bismuth-based triple therapy in *H. pylori* eradication has shown increase cure rate and minimal side effects^(7,8). Probiotic (eg. *Lactobacillus acidophilus* and *Bifidobacterium bifidum*) supplementation to triple therapy for *H. pylori* eradication have been shown to enhance the *H. pylori* eradication rate⁽⁹⁻¹¹⁾. We wanted to search for an optimized combination of drugs and duration for triple therapy that may be effective in Thailand. We therefore, conducted performed this study to assess *H. pylori* eradication with triple therapy plus bismuth with probiotic supplement for 7 or 14 days. The effects of CYP2C19 genotype and antibiotic resistance were also examined.

METHODS

Between December 2012 and December 2013, patients who underwent a gastroscopic examination for dyspeptic symptoms at Thammasat University Hospital, Bangkok, Thailand, were recruited for the studies. Entry criteria included age over 18 and those who had never received *H. pylori* eradication, nor PPI, H₂ receptor antagonists, bismuth and antibiotics in the preceding one month. We also excluded those who were receiving anticoagulants, non-steroid anti-inflammatory drugs, or who had previously undergone gastric surgery, and patients with significant systemic diseases, patients with drug or alcohol abuse, and women who were breast feeding or had child-bearing potential without using effective contraception. Signed informed consent was obtained from all participants. The diagnosis of non-ulcer dyspepsia was made from

symptomatic assessment and endoscopic findings. Dyspeptic patients with normal endoscopy and those with gastritis without erosions or ulcer were considered to have non-ulcer dyspepsia (NUD) and were enrolled in this study. During endoscopy, 4 biopsy samples from the gastric antrum were obtained for rapid urease test, *H. pylori* culture and E-test, GenoType^(®) HelicoDR, histological examination and CYP2C19 genotype. The CYP2C19 genotype divided into 3 groups; rapid metabolizer (RM), intermediate metabolizer (IM) and poor metabolizer (PM), and was performed as previously described⁽¹⁰⁾. The presence of *H. pylori* was defined as: 1) positive *H. pylori* culture; or two positive tests (rapid urease test and histology).

Therapeutic regimens

Eligible subjects were randomized using a computer-generated randomization list. Randomization was made at the time of selection of either 7-day or 14-day triple therapy plus bismuth with probiotic supplement, or placebo (drinking yogurt with or without probiotic preparing by Dutch Mill Co., Ltd, Thailand). The treatment regimen consisted of lansoprazole (30 mg) twice a day, amoxicillin (1 gram) twice a day, long acting clarithromycin MR (1000 mg) once a day, and bismuth subsalicylate (524) 2 tablets twice daily. PPI therapy was limited to 7 or 14 days. Probiotic mixture (one serve) consisted of *Bifidobacterium lactis* $\geq 10^9$ CFU/serve, *Lactobacillus acidophilus* $\geq 10^9$ CFU/serve and *Lactobacillus paracasei* $\geq 10^9$ CFU/serve. Placebo was in the form of drinking yogurt without probiotic.

Post-Therapy follow-up

Patients returned for assessment of eradication response by ¹³C-urea breath test (UBT) at least 2 weeks after completion of therapy. The UBT was performed as previously described⁽⁴⁾. Successful eradication was defined as a negative UBT result. Pill count was conducted and drug consumption over 80% was defined as good compliance. Side effects were assessed by personal interview using open-ended questions and a questionnaire administered by one of the investigators. New symptoms and exacerbation of pre-existing symptoms during the therapy period were considered to be therapy related side effects.

Questionnaire

The questionnaire included personal history of smoking. Smokers were defined as those who con-

sumed more than 1 pack of cigarettes per week. The medical history and underlying diseases (e.g., hypertension, diabetes, hyperlipidemia, coronary artery disease) were recorded. Adverse events evaluated included diarrhea, bitter taste, anorexia, nausea, vomiting, and skin rashes. Those who considered that their symptoms disturbed daily life were defined as having major adverse effects. Symptoms that were not considered by the patient to disturb daily life were defined as minor adverse effects.

Statistical Analysis

Subjects were randomized into 4 parallel groups to prevent selection bias. We expected the eradication rate of triple therapy plus bismuth with probiotic supplement as an empiric therapy to be $\geq 90\%$. Treatment success was prespecified as a cure rate $\geq 95\%$ (i.e. grade A) as described in previous studies⁽¹²⁾ and failure as a cure rate of $<90\%$ per protocol. The demographic information and frequencies of adverse effects were compared using chi-square, Fisher's exact test and student's *t*-test. The *p*-values <0.05 were considered to be statistically significant. The study was conducted according to the good clinical practice guideline as well as the Declaration of Helsinki, and was approved by our local ethics committee. All subjects signed informed consent to participate in this study.

RESULTS

One hundred patients (28 males, 72 females, mean age 50.5 years.) were enrolled and were randomized (25 to each group to receive the 7-day regimen with probiotic supplement or to receive placebo, and another 25 to each of another 2 groups to receive the 14-day regimen with probiotic supplement or to receive placebo. The baseline demographic data were similar consistent with the randomization process (Table 1).

Eradication of *H. pylori* infection and adverse events

One hundred subjects completed the study without any one dropping out. The eradication rate with the 7-day and the 14-day regimens with probiotic supplement achieved the eradication rate of 100%, and better than those without probiotic supplement (100% vs. 92% and 100% vs. 96%, respectively) as demonstrated in Figure 1. Common side effects included diarrhea, bitter taste, anorexia, nausea, vomiting, and skin

Table 1. Baseline demographic and clinical characteristics of patients.

Characteristic data	7-day regimen (n = 50)	14-day regimen (n = 50)
Age	49.7	51.2
Sex	17	11
Male	33	39
Female		
Underlying diseases		
-Cardiovascular diseases	6	4
-Hypertension	26	24
-Dyslipidemia	24	22
Smoking	8	6
Alcohol consumption	6	4

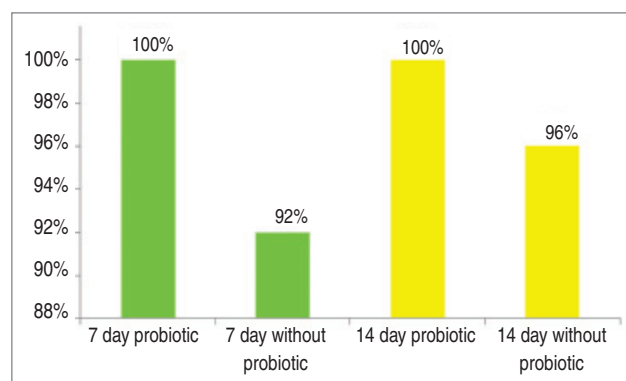


Figure 1. Eradication rate of treatment regimens.

rashes, and were found in all groups. Interestingly, bitter taste was significantly lower in the 7-day regimen with probiotic supplement than those in the placebo group (40% vs. 64%; $p=0.04$) as shown in Table 2. No subject experienced a major adverse event.

Antibiotic susceptibility testing were performed for 87 strains (55 from E-tests, 32 from GenoType[®]) HelicoDR which demonstrated 36.7% of metronidazole resistance and 1.1% clarithromycin resistant strains, as demonstrated in Table 3. CYP2C19 genotype was performed in 98 cases (49 from 7-day and 14-day regimens). The prevalence of CYP2C19 genotype revealed 40.8% RM, 48% IM and 10.2% PM. The prevalence of CYP2C19 genotype was similar in all groups of patients (Table 3).

Table 2. Adverse reactions of subjects in the two pilot studies.

Adverse reactions	7-day probiotic regimen (n = 25)	7-day non-probiotic regimen (n = 25)	p-value	14-day probiotic regimen (n = 25)	14-day non-probiotic regimen (n = 25)	p-value
Bitter taste	8 (40%)	16 (64%)	0.04	10 (40%)	15 (60%)	0.25
Diarrhea	6 (24%)	10 (25%)	0.36	6 (24%)	10 (40%)	0.36
Black stool	24 (96%)	22 (88%)	0.6	21 (84%)	22 (88%)	1.00
Rash	1 (4%)	1 (4%)	0.47	4 (16%)	1 (4%)	0.34
Nausea/Vomiting	6 (24%)	7 (28%)	1.00	5 (20%)	5 (20%)	0.72

Table 3. CYP2C19 genotype, antibiotic resistance and outcome of treatment.

CYP2C19 genotype	% Eradication rate			
	7-day regimen		14-day regimen	
	with probiotic (n=24)	with placebo (n=25)	with probiotic (n=25)	with placebo (n=24)
RM (40.8%)	10 (100%)	11(91%)	9 (100%)	10 (100%)
IM (49%)	11(100%)	10 (90%)	15 (100%)	12 (91.6%)
PM (10.2%)	3 (100%)	4 (100%)	1 (100%)	2 (100%)
*Prevalence of metronidazole resistance	45.8%	40%	36%	25%
*Prevalence of clarithromycin resistance	0	0	4%	0

DISCUSSION

In many countries including Thailand standard triple therapy with a clarithromycin-containing regimen is associated with an eradication rate less than 80% arriving to many factors such as antibiotic resistance especially with clarithromycin^(2-4,13,14). Bismuth has long been known as an anti-*H. pylori* with fewer side effects and without any resistance report⁽²⁾. Administration of bismuth in the quadruple therapy was shown increases the eradication rate to approximately 90% and was better than the triple therapy⁽⁷⁻⁸⁾. Previous studies indicated that probiotic supplementation to standard triple therapy for *H. pylori* eradication significantly enhanced the *H. pylori* eradication rate⁽⁹⁻¹¹⁾. A recent Chinese study demonstrated that adding probiotics before or after standard triple therapy may improve *H. pylori* eradication rates⁽¹⁵⁾. Currently, *H. pylori* eradication with standard triple therapy adding bismuth and probiotic supplement regimens has not been reported.

Our study demonstrated that triple therapy using a 14-day high-dose PPI and long-acting clarithromycin provided an excellent cure rate (100%) regardless of the CYP2C19 genotype⁽¹⁶⁾. From this study, we demonstrated that the 7-day standard triple therapy consisting of only double dose of PPI, amoxicillin, long acting clarithromycin plus bismuth and probiotic supplement also produced an excellent efficacy (100%) for *H. pylori* eradication in our area, which has a high prevalence of metronidazole resistance and low prevalence of clarithromycin resistance. Furthermore, the efficacy of this regimen achieved the high cure rate regardless of CYP2C19 genotype. Adding bismuth and probiotic supplement might be another way to improve the eradication rate of standard triple therapy without increasing the dose and the duration of PPI.

A probiotic is a live microbial organism when ingested, beneficial to human capable of preventing diseases and promoting good health⁽¹⁵⁾. Probiotic supplement, either *Lactobacillus* or *Bifidobacterium* containing drinking yogurt, may have beneficial effects on *H.*

pylori eradication by decreasing *H. pylori* load despite antimicrobial resistance, thus improving the efficacy of eradication therapy^(17,18). Previous studies also demonstrated that adding probiotics may reduce the side effects of the treatment regimens^(19,20). Alterations in intestinal flora were related to anti-*H. pylori* regimen, combining a PPI, clarithromycin or amoxicillin and metronidazole. Probiotics may restore physiology of human intestine, and might prevent or reduce antibiotic-associated symptoms⁽¹⁹⁾.

In summary, our study suggested that the 7-day regimen consisting of PPI, amoxicillin, long acting clarithromycin, plus bismuth and probiotic supplement may be a therapeutic option for *H. pylori* eradication in areas with high rate of metronidazole resistance and low prevalence of clarithromycin resistance. This regimen is also of high efficacy regardless of CYP2C19 genotype, and can be chosen as an alternative first line for *H. pylori* eradication in areas such as Thailand. Adding probiotic could help reduce side effects of the medications. Larger multi-center studies are needed to test this hypothesis.

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