RESEARCH ARTICLE

Improved Eradication Rate of Standard Triple Therapy by Adding Bismuth and Probiotic Supplement for *Helicobacter pylori* Treatment in Thailand

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) remains an important cause of gastric cancer and peptic ulcer disease worldwide. Treatment of *H. pylori* infection is one of the effective ways to prevent gastric cancer. However, 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 to standard triple therapy for *H. pylori* eradication. Materials and Methods: In this prospective single center study, *H. pylori* infected gastritis patients were randomized to receive 7- or 14-day standard triple therapy plus bismuth with probiotic or placebo. Treatment regimen consisted of 30 mg lansoprazole twice daily, 1 g amoxicillin twice daily, 1 g clarithromycin MR once daily and 1,048mg bismuth subsalicylate twice daily. Probiotic bacteria composed of *Bifidobacterium lactis*, *Lactobacillus acidophilus* and *Lactobacillus paracasei*. Placebo was conventional drinking yogurt without probiotic. CYP2C19 genotyping and antibiotic susceptibility tests were also done. *H pylori* eradication was defined as a negative ^{13}C-urea breath test at least 2 weeks after completion of treatment. Results: One hundred subjects were enrolled (25 each to 7- and 14-day regimens with probiotic or placebo). Antibiotic susceptibility tests showed 36.7% metronidazole and 1.1% clarithromycin resistance. CYP2C19 genotyping revealed 40.8%, 49% and 10.2% were rapid, intermediate and poor metabolizers, respectively. The eradication rates of 7- or 14 regimens with probiotics were 100%. Regarding adverse events, the incidence of bitter taste was significantly lower in the 7- day regimen with the probiotic group compared with 7- day regimen with placebo (40% *vs.* 64%; p=0.04). Conclusions: The 7-day standard triple therapy plus bismuth and probiotic can provide an excellent cure rate of *H. pylori* (100%) in areas with low clarithromycin resistance such as Thailand, regardless of CYP2C19 genotype. Adding a probiotic also reduced treatment-related adverse events.

Keywords: Bismuth - triple therapy - *Helicobacter pylori* - probiotic - Thailand

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Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacteria, which has been proven to be etiologically associated with gastric cancer and peptic ulcer disease (Vilaichone and Mahachai, 2001, Vilaichone et al., 2006). The causal relationship between of *H. pylori* and the development of gastric cancer has been documented in both epidemiological and basic research studies (Parsonnet et al., 1991, Uemura et al., 2001, Demirel et al., 2013, Karami et al., 2013, Basiri et al., 2014). From pathogenic standpoint, *H. pylori* can cause chronic atrophic gastritis and intestinal metaplasia, which is considered as precancerous lesions. Therefore, *H. pylori* has been classified as a type I carcinogen by the International Agency for Research in Cancer (IARC, 1994, Abebaw et al., 2014). Moreover, a recent meta-analysis has shown that *H. pylori* eradication potentially reduces the incidence of gastric cancer, especially in the areas where the prevalence of gastric cancer is high (Fock, 2014, Ford et al., 2014).

For years, *H. pylori* has been regarded as a difficult-to-treat infection due to the bacterium's nature, and readily acquired resistance to commonly used antibiotics. Hence, standard triple therapy with proton pump inhibitor (PPI), amoxicillin and clarithromycin is no longer recommended as an empiric choice in most countries (Chey and Wong, 2007, Mahachai et al., 2011). However, triple therapy is still suggested in the areas where the rate of clarithromycin resistance is low, or when the treatment is chosen based on the antibiotic susceptibility testing. Noteworthy, standard triple therapy is adversely affected by many factors besides

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antibiotic resistance, including smoking, the dosage and duration of treatment. For instance, an extended duration of PPI and clarithromycin-containing triple therapy from 7 to 10-14 days improved the eradication rate by about 5% (Malfertheiner et al., 2012). Bismuth-based triple therapy for *H. pylori* eradication has been shown to increase cure rate with minimal side effects (Ford et al., 2008, Fock et al., 2009). Interestingly, recent studies have demonstrated the positive effect of adding probiotic (*eg. Lactobacillus acidophilus and Bifidobacterium bifidum*) to standard triple therapy for *H. pylori* eradication (Mirzaee and Rezahosseini, 2012, Zheng et al., 2013, Wang and Huang, 2014).

In the present study, we aimed at searching for an optimal combination of drugs and duration for *H. pylori* eradication in Thailand. Here, we report a prospective randomized trial evaluating *H. pylori* eradication by using standard triple therapy plus bismuth with or without probiotic supplement for 7 or 14 days. The effects of CYP2C19 genotype and antibiotic resistance were also examined.

Materials and Methods

Patients

All patients age over 18 years who underwent gastroscopic examination at Thammasat University Hospital, for dyspeptic symptoms between December 2012 and December 2013 were assessed for eligibility. After the endoscopy, those with a diagnosis of non-ulcer dyspepsia, which was established in subject with a normal endoscopic finding or mild gastritis, were considered for inclusion in this study. Exclusion criteria were: (1) a history of previous *H. pylori* eradication, (2) those received PPI, H_2-receptor antagonists, bismuth or antibiotics 1 month prior to the inclusion, (3) currently on anticoagulants or non-steroidal anti-inflamamtory drugs, (4) history of gastric surgery, (5) breast-feeding woman, (6) drug or alcohol abuser, and (7) other serious comorbidities (organ failure, malignancies). All patients provided written informed consent.

The diagnosis of H. pylori infection

During the endoscopy, 4 biopsy samples from gastric antrum were obtained for rapid urease test, *H. pylori* culture and Epsilometer test (E-test) or GenoType®HelicoDR, histological examination and CYP2C19 genotype. The CYP2C19 genotype testing was performed according to previously described technique (Zheng et al., 2013), and the results were expressed as: rapid metabolizer (RM), intermediate metabolizer (IM) or poor metabolizer (PM). The presence of *H. pylori* was defined as: 1) positive *H. pylori* culture, or (2) two positive tests (rapid urease test and histology).

Therapeutic regimens

All patients were randomly assigned by using a computer-generated list into 4 groups: (1) 7-day triple-bismuth-probiotic, (2) 14-day triple-bismuth-probiotic, (3) 7-day triple-bismuth-placebo, or (4) 14-day triple-bismuth-placebo. Standard triple therapy consisted of 30mg lansoprazole twice daily, 1 g amoxicillin twice daily and 1g long acting clarithromycin MR once daily. Bismuth was given at a dose of 1048 mg twice daily. Probiotic yogurt composed of *Bifidobacterium lactis*≥10^9 cfu/serve, *Lactobacillus acidophilus*≥10^9 cfu/serve and *Lactobacillus paracasei*≥10^9 cfu/serve, whereas placebo was a conventional yogurt without probiotic. All yogurts were prepared by Dutch Mill Co., Ltd, Thailand.

Questionnaire

The questionnaire included personal history of smoking. Smokers were defined as those who consumed at least 1 pack of cigarettes weekly. The medical histories and underlying diseases (e.g., hypertension, diabetes, hyperlipidemia, and coronary artery disease) were also recorded.

Post-therapy follow-up

At least 2 weeks after completion of treatment, ^{13}C-urea breath test (UBT) was carried out in all patients to assess *H. pylori* eradication. The UBT was performed as previously described method (Mahachai et al., 2011). Successful eradication was defined as a negative UBT result. Pill count was conducted, and drug consumption over 90% 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. The potential adverse events listed in the questionnaires were diarrhea, bitter taste, anorexia, nausea, vomiting, and skin rashes. New symptoms and exacerbation of pre-existing symptoms during the treatment period were considered to be therapy-related adverse events. Serious adverse events were defined as events that disturbed patients' daily life.

Statistical analysis

We expected the eradication rate of triple therapy plus bismuth with probiotic supplement as an empiric therapy to be≥90%. Treatment success was pre-specified as a cure rate of≥95% (i.e. grade A) as described in previous studies (Graham et al., 2007), and failure as a cure rate of<90% per protocol. The demographic characteristics and frequencies of adverse effects were compared using chi-squared, Fisher's exact and student's t-test. The P-value <0.05 was 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.

Results

One hundred patients were enrolled in this study, 28% were men with a mean age of 50.5 years. All 100 patients were randomized in to 4 groups as previously described. As shown in Table 1, the baseline demographic data were similar between those received 7- and 14-day regimen.

Eradication of H. pylori infection

All 100 patients completed the study without any drop out. Therefore, the results analyzed by both intention-to-treat and per-protocol analyses were identical. As

shown in Figure 1, the eradication rates in those received 7-day or 14-day regimen plus probiotic supplement were 100%. However, there was no statistical significance in the eradication rates between those assigned to probiotic and placebo.

Table 1. Baseline Demographic Data of All 100 Patients

Characteristic data	7-day regimen (n=50)	14-day regimen (n=50)
Age (years)	49.7	51.2
Male (%)	34	22
Underlying disease (%)		
Hypertension	52	48
Dyslipidemia	48	44
Cardiovascular disorder	12	8
Smoking (%)	16	12
Alcohol consumption (%)	12	8

*P-value: not significant for all variables

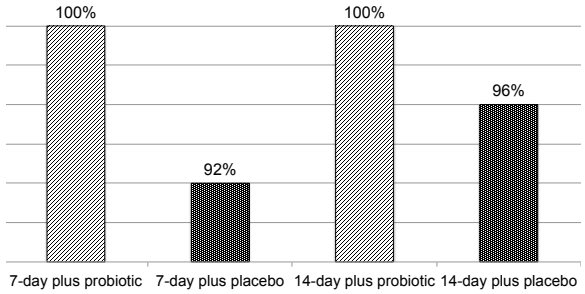

Figure 1. The Eradication Rates According to Treatment Regimens

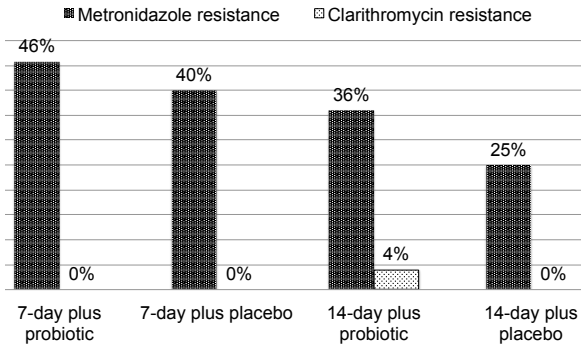

Figure 2. The Prevalence of Antibiotic Resistance Determined by E-test (n=55) and Genotype®HelicoDR (n=32)

As shown in Figure 2, antibiotic susceptibility tests were performed in 87 strains (55 from E-test and 32 from GenoType®HelicoDR), which demonstrated 36.7% of metronidazole resistant and 1.1% of clarithromycin resistant strains. CYP2C19 genotype tests were performed in 98 cases (49 from each 7-day and 14-day regimens). The CYP2C19 genotype tests revealed 40.8% RM, 48% IM and 10.2% PM. The prevalence of CYP2C19 genotype was similar in all groups of patients (Table 2).

Adverse events

The common side effects included diarrhea, bitter taste, anorexia, nausea, vomiting, and skin rashes, which were found in all groups. All documented adverse reactions are shown in Table 3. Interestingly, the incidence of bitter taste was significantly lower in 7-day regimen with probiotic supplement than those with placebo group (40% *vs*. 64%; p=0.04). No subject experienced any major adverse event.

Discussion

Gastric cancer is the third most common cause of cancer-related death globally, thus it has been considered as an important public health burden (Hajmanoochehri et al., 2013, Ford et al., 2014, Rahman et al., 2014). Moreover, the prognosis of affected patients is usually poor due to the fact that most cases are diagnosed after age 50 and presented at advanced stage (Lin et al., 2014, Rahman et al., 2014). Therefore, several efforts have focused on how to prevent the development of gastric cancer. Currently, several epidemiological and experimental data support a pathological role between *H. pylori* and the development of gastric cancer (Parsonnet et al., 1991, Uemura et al., 2001, Demirel et al., 2013, Karami et al., 2013, Basiri et al., 2014). At the present time, *H. pylori* infects more than 50% of the global population mainly in the developing countries (Abebaw et al., 2014). It was estimated that *H. pylori* infection accounts for approximately 650,000 new cases of non-cardiac gastric cancer annually (de Martel et al., 2012). Indeed, consensus groups from Asia and Europe have recommended *H. pylori* eradication as primary prevention in population with high incidence of gastric cancer (Fock et al., 2009, Malfertheiner et al., 2012).

The eradication rate of *H. pylori* by standard triple

Table 2. Results of CYP219 Genotypetesting and Eradication rate (Shown in Parentsese) According to Treatment Regimens

CYP2C19 genotype (n=98)	7-day plus probiotic (n=24)	7-day plus placebo (n=25)	14-day plus probiotic (n=25)	14-day plus placebo (n=24)
RM (n=40; 40.8%)	10 (100%)	11 (91%)	9 (100%)	10 (100%)
IM (n=48; 49%)	11 (100%)	10 (90%)	15 (100%)	12 (91.6%)
PM (n=10; 10.2%)	3 (100%)	4 (100%)	1 (100%)	2 (100%)

*number in parenteses are eradicationa rates

Table 3. Adverse Events According to 4 Treatment Regimens

Adverse events	7-day plus probiotic (n=25)	7-day plus placebo (n=25)	P-value	14-day plus probiotic (n=25)	14-day plus placebo (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
Rash	1(4%)	1(4%)	0.47	4(16%)	1(4%)	0.34
Nausea/vomiting	6(24%)	7(28%)	1	5(20%)	5(20%)	0.72

therapy with clarithromycin-containing regimen was reported to be less than 80% in many countries worldwide, including Thailand, due to many factors such as, antibiotic resistance, especially clarithromycin-resistant strain (Vilaichone et al., 2006, Graham, 2009, Mahachai et al., 2011, Rimbara et al., 2011). Bismuth has long been known as an anti-*H. pylori* drug with minimal side effects. So far, *H. pylori* strains resistant to bismuth have not been reported (Vilaichone et al., 2006, Malfertheiner et al., 2012). Several studies showed that an administration of bismuth in quadruple therapy increased the eradication rate of *H. pylori* to approximately 90%, significantly better than of standard triple therapy (Ford et al., 2008, Fock et al., 2009). In addition, recent studies have demonstrated that adding probiotic supplement to standard triple therapy significantly enhanced the *H. pylori* eradication rate (Mirzaee and Rezahosseini, 2012, Zheng et al., 2013, Wang and Huang, 2014). Thus far, the efficacy of *H. pylori* eradication by adding bismuth and probiotic to standard triple therapy has not been reported.

Our previous 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 (Prasertpetmanee et al., 2013). In the present study, we demonstrated that 7-day standard triple therapy consisting of a double dose of PPI, amoxicillin, long acting clarithromycin plus bismuth and probiotic supplement also resulted in an excellent efficacy (100%) for *H. pylori* eradication in this region, which has a high prevalence of metronidazole resistance and low prevalence of clarithromycin resistance. Furthermore, the exceptional high cure rate was maintained regardless of CYP2C19 genotype. Adding bismuth and probiotic supplement might be a good option to improve the eradication rate by standard triple therapy apart from increasing the dosage and duration of PPI.

Probiotics are live microbial organisms that, when consumed, provide a beneficial effect on human health, especially on the digestive tract and immune system (Fuller, 1991, Otles et al., 2003). Data coming from our and previous studies show that probiotic supplement either *Lactobacillus* or *Bifidobacterium* containing drinking yogurt enhances the *H. pylori* eradication. This positive result could possibly be explained by probiotics decrease *H. pylori* load, in spite of antimicrobial resistance, thus improving the efficacy of eradication therapy (Sheu et al., 2006, Du et al., 2012). In additions, as shown in our study, adding probiotics also reduced the side effects of the treatment regimens (Armuzzi et al., 2001, Armuzzi et al., 2001). The other possible explanation is standard anti-*H. pylori* regimen that combines PPI, clarithromycin or amoxicillin and metronidazole could alter the equilibrium of intestinal floras. Accordingly, adding probiotics may restore physiology of human intestine, and might prevent or reduce antibiotic-associated adverse symptoms (Armuzzi et al., 2001).In summary, our study showed that the 7-day standard triple therapy might be reemerged into clinical practice for *H. pylori* eradication by adding bismuth and probiotic supplement, especially in the areas with low prevalence of clarithromycin resistance. This regimen also provides an excellent efficacy regardless of CYP2C19 genotype, and could be used as an alternative first line for *H. pylori* eradication in this area. Our study also demonstrated that adding probiotic reduced the side effects related to the medications during treatment. However, larger multi-center controlled studies are needed to confirm this hypothesis.

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