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A Triple and Quadruple Therapy with Doxycycline and Bismuth for First-Line Treatment of *Helicobacter pylori* Infection: A Pilot Study

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Keywords
Helicobacter pylori infection, first-line therapy, quadruple therapy, amoxicillin, DOX, bismuth subcitrate.

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Abstract

Background: Tetracycline-containing triple therapy has been suggested as an alternative first-line therapy for *H. pylori* infection.

Aim: To evaluate the effect of two dosages of doxycycline (DOX) associated with amoxicillin and esomeprazole with and without bismuth subcitrate as first-line treatment of *H. pylori* infection.

Methods: *Helicobacter pylori*-positive patients underwent a 10-day therapy randomized into four groups: Group A received esomeprazole, amoxicillin, and DOX-100 mg b.i.d. (EAD-100), Group B a quadruple therapy with esomeprazole, amoxicillin, DOX-100 mg b.i.d. and bismuth subcitrate (EADB-100), Group C a triple therapy with esomeprazole, amoxicillin, and DOX-200 mg b.i.d. (EAD-200) and Group D a quadruple therapy with esomeprazole, amoxicillin, DOX-200 mg b.i.d., and bismuth subcitrate (EADB-200). Success was accessed by ^{13}C urea breath test 2 months after the end of treatment. The number of patients to be recruited for each group had to be at least 50 subjects. Treatment success of 80% or less was consid- ered unacceptable. Stopping rules therefore were anytime six failures had occurred.

Results: In the EAD-100 group and in EAD-200 group, the recruitment was stopped at the 14th and 15th patient, respectively. Fifty-two patients entered in the EADB-100 group and 51 in the EADB-200 group. Intention to treat eradication was in EADB-100 group 46/52 (88.5%, 95% CI 76.6–95.6); inthe EADB-200 group 47/51 (92.1%, 95% CI: 81.1–97.8) (n.s.). Side effectswere absent.

Conclusion: The adjunction of bismuth subcitrate to a triple therapy that includes esomeprazole, amoxicillin, and DOX in patients who are treated forthe first time for the *H. pylori* infection potentiates the therapeutic effect. This regimen, however, deserves to be optimized in terms of duration and dose of DOX.

Helicobacter pylori has been involved in the development of chronic gastritis and peptic ulcer disease and has been linked to the pathogenesis of gastric lymphoma and gastric cancer; hence, it is recommended that this infection must be cured whenever it is diagnosed [1–3]. *Helicobacter pylori* is susceptible to several antibiotics including clarithromycin, amoxicillin, metronidazole, tinidazole, tetracycline, rifabutin, and fluoroquinolones [4,5]. This bacterial infection, however, has proven challenging to cure. Antibiotics have been useful in the treatment of *H. pylori*-related diseases. However, *H. pylori* may be resistant in various degree to one or more of the above-cited antibiotics even in those subjects never treated specifically for the infection, and antibiotic resistance may become a key factor for treatment failure [6]. Resistance rates vary in different geographic areas, and therefore, the selection of therapeutic regimes needs adjustments according to local resistance pattern if avail- able [7,8]. The prevalence of antibiotic resistance in var- ious regions is correlated with general use of antibiotic

in the each region [9,10]. In our region, the resistance rate of *H. pylori* to amoxicillin, clarithromycin, metronidazole, moxyfloxacin, levofloxacin and tetracycline is 1.37%, 60.27%, 39.72%, 37%, 38%, and 2.74%, respectively (Marzio L, Ciccaglione AF, Cellini L. Unpublished results).

Several mechanisms are involved in the development of resistance. *Helicobacter pylori* eradication failures may be due to acquiring chromosomal mutations or by acquisition of foreign genes carried on mobile genetic elements (horizontal gene transfer) that cause changes in each drug's site action [9,11] and cannot be reserved by increasing the dose or duration [12]. The lack of patient compliance is assumed to be a key factor in eradication failure, when adverse events occur, which are relatively frequent, leading to treatment discontinuation [13,14]. Insufficient antibiotic concentration at the site of infection contributes to the spreading of resistant strains [13,14].

Classical triple therapies with proton-pump inhibitors (PPI), clarithromycin, and amoxicillin, or metronidazole are the mainstay of current treatment, but resistance to clarithromycin has been reducing its effectiveness in recent years with an eradication rate below 80% of treated cases [15,16]. In geographic areas with high clarithromycin resistance, bismuth-containing quadruple therapy is superior to standard triple therapy. The original quadruple therapy containing omeprazole, bismuth subcitrate, metronidazole, and tetracycline achieves higher eradication rates compared with the standard triple therapy [17,18]. In a study randomized, open-label, phase 3 trial, a quadruple regimen with a capsule containing bismuth citrate potassium, metronidazole, and tetracycline given with omeprazole was found more efficacious than a clarithromycin-containing triple therapy in patients never treated in their past for the infection[19].

Selection of first-line eradication therapy is important in avoiding primary failure [20]. Some recent works have focused on the of eradication therapy [21,22] and compared various available eradication regimens.

In an Iranian study, an eradication rate of 68% was achieved using the following regime: DOX 100 mg b.i.d., co-amoxiclav 625 mg t.i.d., and omeprazole 20 mg b.i.d. for a duration of 2 weeks, suggesting that could be useful in first-line *H. pylori* eradication [23]. As a synthetic antibiotic in the tetracycline class, DOX has attracted much attention as a possible agent for *H. pylori* eradication. DOX is a widely used tetracycline antibiotic in several infections. With respect to tetracycline, DOX has a simpler dosing schedule, leading to a better compliance in patients undergoing eradication therapies and is more easily absorbed with food. Furthermore, Heep et al. [24] have found no secondary resistance to DOX in *H. pylori* isolates from patients in which failed one or more eradication therapies.

The aim of the study has been to compare DOX-containing triple therapy at 100 mg b.i.d. or at 200 mg b.i.d. versus DOX at 100 mg b.i.d. or at 200 mg b.i.d. and bismuth-containing quadruple therapy for first-line treatment of *H. pylori* infection.

Materials and Methods

Eligibility Criteria

Patients were enrolled among those with a positive ^{13}C urea breath test performed with citric acid and 75 mg of ^{13}C urea (UBT) within 10 days prior to the start of the study. Exclusion criteria included age: <18 or >80 years; any previous treatment for *H. pylori* infection, treatment with PPI (omeprazole, lansoprazole, pantoprazole, rabeprazole, esomeprazole), H2-blockers (ranitidine, nizatidine, cimetidine, famotidine, roxatidine), and/or antibiotics during the 4 weeks before the study; gastrointestinal malignancy; severe concomitant diseases; previous gastric surgery. All patients enrolled in the study were affected by chronic dyspepsia without alarm symptoms. Only patients older than 55 years underwent an upper endoscopy before the admission to the study. Patients with active gastric and/or duodenalulcer or gastric neoplasia were excluded [25].

Study Protocol

Consecutive *H. pylori*-positive patients never treated in their past for the infection were randomized into four groups with different therapeutics regimens. A simple randomization schedule was used following a computer-dedicated software [26]. Group A included patients treated with a triple therapy with esomeprazole, amoxicillin, and DOX 100 mg b.i.d. (EAD-100). Group B included patients treated with a quadruple therapy with esomeprazole, amoxicillin, and DOX 100 mg b.i.d. and bismuth subcitrate (EADB-100). Group C included patients treated with a triple therapy with esomeprazole, amoxicillin, and DOX 200 mg b.i.d. (EAD-200). Group D included patients treated with aquadruple therapy with esomeprazole, amoxicillin, and DOX 200 mg b.i.d. and bismuth subcitrate (EADB-200). All four groups were treated with these therapeutic agents for 10 days.

While esomeprazole, amoxicillin, and DOX were administered before breakfast and supper, bismuth salt was administered 3 hours later as it may bind with DOX and prevent its full absorption [27].

Dosages and time of administration of the studied drugs are summarized in Tables 1 and 2.

Patients were informed that bismuth renders the stools a black dark color.

Helicobacter pylori eradication was defined as a negative result in UBT performed at least 8 weeks after the end of treatment with a delta over baseline (DOB)value equal or <5.

Statistical Analysis

For this study, the number of patients to be recruited for each of the four groups had to be at least 50 sub-jects. An effective therapy was defined as a per-protocol

treatment success of 90% or greater, treatment success of 80% or less was prospectively deemed unacceptable.

Stopping rules were anytime six failures had occurred

[28].

Statistic evaluation was carried out using the chi-

square analysis. A *p*-value of .05 or less was considered statistically significant. Per protocol (PP) in which only data from adherent subjects are analyzed, and intention to treat (ITT) in which all subjects are followed regardless of adherence, was calculated.

Results

Patient Characteristics are Summarized in Tables 1 and 2.

Six treatment failures were reached in the EAD-100 group and in the EAD-200 group at the fourteenth and

Table 1 Summary of study data

Triple therapy regimen (EAD-100)	Quadruple therapy regimen (EADB-100)
Esomeprazole (ESO) 20 mg b.i.d. (8.00 a.m.–8.00 p.m.)	ESO 20 mg b.i.d. (8.00 a.m.–8.00 p.m.)
Amoxicillin (AMO) 1 g b.i.d. (8.00 a.m.–8.00 p.m.)	AMO 1 g b.i.d. (8.00 a.m.–8.00 p.m.)
Doxycycline (DOX) 100 mg b.i.d. (8.00 a.m.–8.00 p.m.)	DOX 100 mg b.i.d. (8.00 a.m.–8.00 p.m.) Bismuth subcitrate 240 mg b.i.d. (11.00 a.m.–11.00 p.m.)
Number of patients: 14	Number of patients: 52
Female/male: 10/4	Female/male: 32/20
Age: mean 49, range 23–75	Age: mean 50, range 20–72
Smoking habits (7 F; 3 M)	Smoking habits (22 F; 15 M)

Table 2 Summary of study data

Triple therapy regimen (EAD-200)	Quadruple therapy regimen (EADB-200)
Esomeprazole (ESO) 20 mg b.i.d. (8.00 a.m.–8.00 p.m.)	ESO 20 mg b.i.d. (8.00 a.m.–8.00 p.m.)
Amoxicillin (AMO) 1 g b.i.d. (8.00 a.m.–8.00 p.m.)	AMO 1 g b.i.d. (8.00 a.m.–8.00 p.m.)
Doxycycline (DOX) 200 mg b.i.d. (8.00 a.m.–8.00 p.m.)	DOX 200 mg b.i.d. (8.00 a.m.–8.00 p.m.) Bismuth subcitrate 240 mg b.i.d. (11.00 a.m.–11.00 p.m.)
Number of patients: 15	Number of patients: 51
Female/male: 10/5	Female/male: 29/22
Age: mean 47, range 24–72	Age: mean 49, range 23–70
Smoking habits (5 F; 2 M)	Smoking habits (15 F; 12 M)
Alcohol consumption (≤30 gr/week; 4 F; 5 M)	Alcohol consumption (≤30 gr/week; 20 F; 18 M)
Follow-up loss: 0	Follow-up loss: 1
Urea Breath Test (UBT) Negative: 9	UBT Negative: 46
UBT Positive: 6	UBT Positive: 3

fifteenth patient recruited, respectively. All patients in each group returned to control; therefore, PP and ITT were coincident. Eradication was 8/14 (57.1%, 95% CI: 28.9–82.3) in the EAD-100 group; 9/15 (60.0%, 95% CI: 32.3–82.7) in the EAD-200 group.

Fifty-two and 51 patients were admitted in EADB-100 and EADB-200 groups, respectively. In both groups, one patient did not return to control. PP eradi- cation was 46/51 (90.1%, 95% CI: 78.6–96.7) and 47/50 (94.0%, 95% CI: 83.5–98.7), respectively. ITT eradi- cation was 46/52 (88.5%, 95% CI: 76.6–95.6) in the EADB-100 group; 47/51 (92.1%, 95% CI: 81.1–97.8) in the EADB-200 group.

Helicobacter pylori eradication rates were significantly higher in the EADB-100 group versus the EAD-100 group in both PP ($p = .003$) and ITT analyses ($p = .007$), and in the EADB-200 group versus the EAD-200 group in both PP ($p = .0008$) and ITT analysis($p = .002$) (Fig. 1).

Adverse Effects

Both treatments were well tolerated with no reported side effects.

Alcohol consumption (≤30 gr/week; 6 F; 4 M)	Alcohol consumption (≤30 gr/week; 24 F; 15 M)
Follow-up loss: 0	Follow-up loss: 1
Urea Breath Test (UBT) Negative: 8	UBT Negative: 46
UBT Positive: 6	UBT Positive: 5

Discussion

Antibiotics have been useful in the treatment of *H. pylori*-related gastroduodenal diseases. Therefore, eradication is considered as an obvious choice. However, the rate of eradication failure has dramatically

risen in many countries due to resistance to antibiotics. As the worldwide increase in the rate of antibiotic resistance represents a problem of relevance, some studies have been performed to identify highly active and well-tolerated anti-*H. pylori* therapies. The constant development of new antibiotic resistances suggests that future treatment will need to be individualized on the basis of host polymorphisms, antibiotic resistance, and demographic factors. Triple therapy containing on a PPI combined with clarithromycin and amoxicillin and/or metronidazole has been the established first-line therapy for *H. pylori* infection over the past years around the world [29,30]. However, the efficacy of standard triple therapy needs to be reconsidered in areas with a high prevalence of clarithromycin- or metronidazole-resistant *H. pylori* strains. The geographic prevalence of antibiotic resistance should influence the choice ofa first-line regimen for the treatment of infection by *H. pylori*. Alternatively, new regimens including sequential, concomitant quadruple, and quadruple therapy.

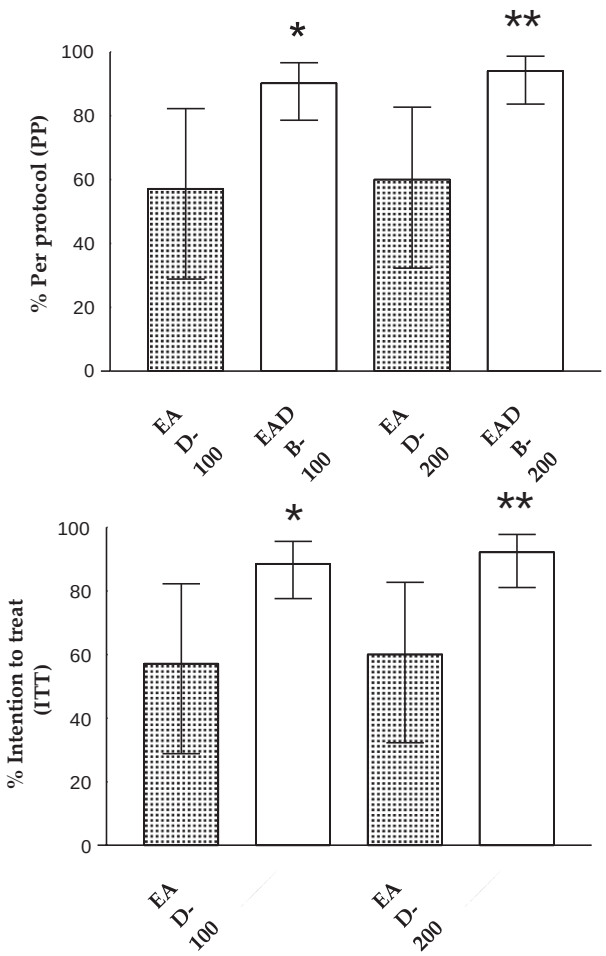

Figure 1 PP and ITT analysis (mean; 95% CI) for eradication rate in 14 patients treated with EAD-100, 52 patients treated with EADB-100, 15 patients treated with EAD-200 and 51 patients treated with EADB-200. PP: *$p < .003$ versus EAD-100; PP: **$p < .0008$ versus EAD-200. ITT: *$p < .007$ versus EAD-100; ITT: **$p < .002$ versus EAD-200.

Tetracycline is an antibiotic that is commonly usedto eradicate *H. pylori* infection in several second-line regimens. The bactericidal activity of tetracycline is a result of the drug's ability to prevent the synthesis of nascent peptide chains via binding to the 30-S ribosomal subunit, as well as blocking the binding of aminoacyl-*tRNA* [31]. The best-studied resistant mechanism has been mostly associated with de novo mutations in the 16S *rRNA* gene, which is based on a single, double, or triple base-pair substitution in adjacent 16S *rRNA* gene [32].

Doxycycline is a synthetic antibiotic in the tetracy- cline class used for several infections which has bacte-riostatic properties through the inhibition of bacterial protein synthesis [33]. Heep et al. [24] found 0% resistance to DOX, in a study to determine secondary resistance in *H. pylori,* isolated from patients in which one or more therapies for the eradication of *H. pylori* had failed.

The therapies containing tetracycline or DOX have been used in the therapy of *H. pylori* infection with variable results.

Malfertheiner et al. have shown that a quadruple therapy with omeprazole and a single three-in-one capsule containing bismuth citrate potassium, metronidazole, and tetracycline for 10 days when compared with a triple therapy for 7 days with omeprazole, amoxicillin, and clarithromycin produces an eradication rate of 80% versus a 55% in the standard therapy group. The authors concluded that quadruple therapy needs to be considered as first-line therapy in areas with a high prevalence of clarithromycin-resistant *H. pylori* strains [19].

In some previous trials, DOX-containing therapies have been variously used, always in triple drug combinations.

Doxycycline-containing therapy has been used in the eradication of *H. pylori* as first-line therapy, as sec- ondline therapy, and as third-line rescue therapy. In those studies that used DOX as first-line therapy for the eradication of *H. pylori*, the results were not satisfactory, with eradication rates ranging from 0 to 68% [23,34–36]. In one study only, higher eradication rates could be reached using a unconventional quadruple therapy with DOX associated with levofloxacin, the sinthetic antiprotozoal nitazoxanide, and a PPI as first-line therapy. In this specific study, the eradication rate was 90% [37]. As second-and third-line therapy, a DOX

quadruple regimen with PPI, amoxicillin, and bismuth salts was used for the treatment of *H. pylori* infection. In these studies, eradication rates were more satisfactory with values ranging between 66.3% and 90% ofthe treated case [38–41].

All the above-cited studies used DOX at the dose of 100 mg b.i.d. for 10 days while to our knowledge, our study is the first to use DOX at 200 mg b.i.d. for 10 days, a dosage that was well accepted by all patients and without side effects. The 200 mg b.i.d., however, does not seem to be more efficacious than the 100 mg b.i.d. with a slight increase in the percentage of eradication. The different results obtained when DOX is used as first-line therapy suggest that there may be a role for DOX in the treatment of *H. pylori* infection; however, optimization in terms of doses and durations needs to be further evaluated. A consistent increase in the eradication rate indeed is obtained when DOX, either with the 100 mg b.i.d. or with the 200 mg b.i.d., is used in association with bismuth subcitrate.

Bismuth exerts its antibacterial action by decreasing mucin viscosity, by binding toxins produced by *H. pylori*, and by preventing bacterial colonization and adherence to gastric epithelium [42,43]. In addition, bismuth reduces the bacterial load and has a synergistic effect with several antibiotics [44].

The results obtained in this study confirm that quadruple regimes, including a PPI, amoxicillin, DOX,and bismuth subcitrate, may constitute an effective first-line option for the treatment of *H. pylori* infection. In addition, the quadruple regime used in the present investigation obtained excellent compliance from all the enrolled patients.

Criticism for our study might include the absence of a preliminary susceptibility test. It may be observed, however, that the culture and sensitivity test was not performed as the resistance of *H. pylori* to amoxicillin and tetracycline in patients never treated is in our region quite low with percentages ranging between 0.1% and 2% of tested strains [45], reducing to a mini- mum possibility to identify a resistant strain to the above-cited antibiotics in our patients. Nevertheless, theknowledge of the organism's antibiotic susceptibility does not necessarily lead to eradication in vivo. Also, as reviewed by Gisbert, [46] even after culture-guided res- cue treatments, the lowest eradication rates were obtained in patients with *H. pylori* strains susceptible to all antibiotics, thereby indicating that other factors, different from in vitro antibiotic susceptibility, influence eradication rates.

Once *H. pylori* is introduced in the stomach, it comes into contact with the mucin layer that covers the epithelial cells, resulting in a strain-dependent adherence between mucin and *H. pylori* [47]. As reported by Cole et al., [48] increasing concentrationsof mucin significantly enhanced planktonic growthover biofilm formation, suggesting that *H. pylori* exists primarily as a biofilm in the environment, but rapidly proliferating as free-living bacteria upon encountering mucin in the human stomach. Biofilm recalcitrant to antimicrobial compounds has long been thought to be due to restricted antibiotic diffusion within the extra- cellular polymeric substances matrix [49]. Antibiotics have been shown to penetrate biofilm readily in some cases and poorly in others, depending on the particular agent and biofilm [50]. As mentioned earlier, the bis- muth exerts its antibacterial action by decreasing mucin viscosity, by binding toxins produced by *H. pylori*, and by preventing bacterial colonization and adherence to gastric epithelium [36,37]. Therefore, the addition of bismuth to the triple therapy helps to overcome *H. pylori* resistance to DOX: only eight patients (five in the EADB-100 group and three in the EADB-200 group) were positive at ^{13}C urea breath test after treatment with quadruple therapy. These data support the hypothesis that the rate of eradication of *H. pylori* infection with a DOX-containing quadruple therapy for 10 days is scarcely influenced by amount of DOX used, that is 100 or 200 mg b.i.d., but more importantly by the inclusion of bismuth. Further studies, however, are needed to optimize the dose of DOX and the duration of treatment.

It may be concluded therefore that the 10-day quadruple therapy consisting of a PPI, bismuth, amoxicillin, and DOX could be recommended as the first-line treatment of *H. pylori* infection in all areas where bismuth is available and particularly in those regions with high clarithromycin, metronidazole, and fluoroquinolones resistance.

Acknowledgements and Disclosures

Competing interest: no competing interest declared.

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