Combinations containing amoxicillin–clavulanate and tetracycline are inappropriate for *Helicobacter pylori* eradication despite high *in vitro* susceptibility

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**Key words**

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**Abstract**

**Background:** The purpose of the present paper was to evaluate the efficacy and tolerability of amoxicillin–clavulanate and tetracycline-based quadruple therapy as an alternative second-line treatment for *H. pylori* infection.

**Methods:** The study subjects consisted of 54 patients infected with *H. pylori*, in whom initial triple therapy had failed. Subjects were randomized to receive the following 7-day therapies: (i) pantoprazole 40 mg b.i.d., tripotassium dicitrate bismuthate 300 mg q.i.d., amoxicillin–clavulanate 1000 mg b.i.d., and tetracycline 500 mg q.i.d. (PBAT); or (ii) pantoprazole 40 mg b.i.d., tripotassium dicitrate bismuthate 300 mg q.i.d., metronidazole 500 mg t.i.d., and tetracycline 500 mg q.i.d. (PBMT). Eradication rates based on antibiotic susceptibility, drug compliance and side-effect rates were evaluated and compared.

**Results:** The *H. pylori* eradication rates were 16.0%/17.4% with PBAT and 65.5%/70.4% with PBMT by intention-to-treat (*P* < 0.001) and per-protocol analyses (*P* < 0.001), respectively. In patients who received PBAT, the eradication rates were only 16.7% (2/12) for both amoxicillin and tetracycline-susceptible *H. pylori* strains. Drug compliance and side-effect rates were similar in the two groups.

**Conclusions:** Despite high individual *in vitro* antimicrobial activity, amoxicillin–clavulanate and tetracycline-based quadruple therapy showed low eradication rates, which strongly suggests that it not be considered as a therapeutic option for *H. pylori* eradication.

**Introduction**

*Helicobacter pylori* is a recognized etiologic agent in peptic ulcer disease, chronic gastritis, gastric mucosa-associated lymphoid tissue lymphoma, and probably in gastric cancer.† Therefore, its eradication is now generally accepted to be the main strategic target against such diseases. To eradicate *H. pylori*, worldwide consensus conferences have recommended the use of triple therapy consisting of a proton pump inhibitor and two antibiotics, usually amoxicillin and clarithromycin, as a first-line therapy and metronidazole-containing quadruple therapy as a second-line rescue therapy.‡ Several meta-analyses have shown that most commonly used first-line therapies fail in up to 15–20% of patients, who then require second-line therapy.‡ However, the standard second-line quadruple therapy is also associated with eradication failure in approximately 20–35% of cases.§ The main causes of its treatment failure are believed to be poor patient compliance and bacterial resistance.¶ Regarding bacterial resistance, high resistance rates of *H. pylori* to metronidazole have been reported worldwide, especially, in Korea and this appears to be increasing.† This high rate of resistance to metronidazole had made the efficacy of metronidazole-containing quadruple therapy limited as a second-line therapy. In fact, recent Korean reports have revealed that the eradication rates of metronidazole-containing second-line therapies are lower than 80% by per-protocol (PP) analysis.¶ Currently, the standard second-line therapy is being questioned in Korea, and we believe that it is time that a standard second-line eradication therapy be re-established for *H. pylori* infection. However, the optimal rescue regimen that should be adopted after a first-line eradication failure remain controversial.

It has been suggested that amoxicillin could be included again in *H. pylori* eradication regimens after initial or second-line failure.
because it has some advantages in terms of efficacy, safety, and negligible primary or acquired resistance.18 However, even in the case of amoxicillin, a recent Korean study reported that the prevalence of primary antibiotic resistance is 18.5%.11 Therefore, the replacement of metronidazole with amoxicillin in the quadruple regimen may not result in optimal H. pylori eradication rates in Korea.

Recent studies have demonstrated that β-lactamase inhibitors, including amoxicillin–clavulanate, enhance the in vitro antibacterial effect against H. pylori.19,20 In addition, amoxicillin–clavulanate showed higher H. pylori eradication rates than amoxicillin alone in two clinical studies.21,22 These findings suggest that amoxicillin–clavulanate-based regimens are good candidates for the treatment of H. pylori infection. Based on this, we undertook the present study to investigate anti-H. pylori therapies by using amoxicillin–clavulanate instead of metronidazole in the standard quadruple regimen. Thus, our aim was to evaluate the efficacy and tolerability of amoxicillin–clavulanate and tetracycline-based quadruple therapy as a second-line treatment for H. pylori infection.

Methods

Patients

We consecutively enrolled, at Seoul National University Hospital and Seoul National University Bundang Hospital in Korea, peptic ulcer patients in whom a first eradication trial with proton pump inhibitor–amoxicillin–clarithromycin combination had failed to eradicate H. pylori infection. The following patients were excluded from the study: those suffering from concurrent critical illnesses, those with a history of previous upper gastrointestinal surgery, those with frequent intake of non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants or steroids, or those in whom the study medication was contraindicated (i.e. pregnant or breast-feeding women, or those with an allergy to the study medications). Other exclusion criteria included the use of antimicrobials and any condition associated with poor compliance, for example drug abuse and alcoholism. All patients enrolled in the study were aware of the study protocol and agreed to comply with the follow-up schedule, and all patients provided written informed consent. The study was approved by the Ethics Committee of Seoul National University Hospital and Seoul National University Bundang Hospital.

Methods

Patients were considered H. pylori-positive if H. pylori was demonstrated by histology, or by rapid urease or 13C-urea breath testing within 3 months prior to inclusion into the study. Patients were randomly assigned to one of the following two second-line treatment regimens: (i) pantoprazole 40 mg b.i.d., tripotassium dicitrate bismuthate 300 mg q.i.d., amoxicillin–clavulanate 1000 mg b.i.d., and tetracycline 500 mg q.i.d (PBAT); or (ii) pantoprazole 40 mg b.i.d., tripotassium dicitrate bismuthate 300 mg q.i.d., metronidazole 500 mg t.i.d., and tetracycline 500 mg q.i.d. (PBMT). Before treatments, enrolled patients were invited to provide cultures for H. pylori by endoscopy.

One week after completing therapy, drug compliance was evaluated by a physician by questioning and pill count. Good drug compliance was considered as a drug intake exceeding 80%. Patients were interviewed for adverse events at the same time. Five weeks after the completion of therapy, patients were diagnosed as having H. pylori eradication if three tests (i.e. histology, rapid urease test, and 13C-urea breath test) were negative.

Rapid urease test

Two biopsy specimens were taken from the prepyloric antrum and midbody by endoscopy and subjected to rapid urease testing (CLOtest, Ballard Medical Products, Draper, UT, USA). The CLOtest was defined as positive when the color change from yellow to red occurred within 24 h.

Helicobacter pylori culture and antimicrobial susceptibility testing

A second set of biopsy specimens was taken along the greater and lesser curvature of the antrum and body, respectively, and four specimens were ground using a homogenizer. The methods used for H. pylori culture and subculture for antimicrobial susceptibility testing were as described previously.11 Resistance breakpoints for amoxicillin, metronidazole, and tetracycline were defined as 0.5≥, 82, and 4≥ µg/mL, respectively.

Histology

A third set of two biopsy specimens was taken from the antrum and body, fixed in 10% buffered saline, and embedded in paraffin. Sections were stained with hematoxylin–eosin for histological evaluation and with modified Giemsa to determine H. pylori status. Specimens were assessed for the presence, type, and density of inflammatory infiltrate and for the presence of H. pylori.

13C-urea breath test

Patients were fasted for 4 h before this test. No test meal was given and a predose breath sample was obtained. Seventy-five mg of 13C-urea powder (Helikit, Isotechnika, Edmonton, Canada) dissolved in 50 mL of water was administered orally. The second breath sample was collected at 30 min. A cut-off value of 4‰ was used. Collected samples were analyzed based on isotope ratios by mass spectrometry (Heliview, Medichems, Seoul, Korea).

Statistical analysis

Helicobacter pylori eradication efficacy was analyzed on an intention-to-treat (ITT) basis, and included all patients enrolled in the study, and on a PP basis (excluding patients with poor compliance to therapy and those with unevaluable data after therapy). The 95% confidence intervals for differences in the eradication rates of the two groups were calculated using standard methods. Continuous variables were analyzed using the Student’s t-test and categorical variables were analyzed using the χ2-test or Fisher’s exact test. Analyses were performed using SPSS (version 11.0; SPSS, Chicago, IL, USA). P < 0.05 was considered to be statistically significant.

To calculate sample size, we assumed a theoretical H. pylori eradication rate of 95% for PBAT18 and an eradication rate of 75%
for PBMT by PP analysis. On this basis, 61 cases per group were necessary to demonstrate significant differences between groups. A preliminary analysis involving approximately 50 patients was conducted as a prelude to a larger study. However, the results of that preliminary analysis prompted us to terminate the intended study at the end of February 2005. Thus, the results presented here are those of the intended preliminary study.

**Results**

**Patient population**

Fifty-four patients were enrolled from May 2004 through February 2005. Of these, 25 patients were randomized to the PBAT group and 29 patients to the PBMT group. Baseline characteristics were similar in these two treatment groups (Table 1). All of these patients were included in the ITT analysis, but four patients were excluded from the PP analysis due to protocol violation (discontinuation of medication due to adverse events). Thus, 23 patients in the PBAT group and 27 patients in the PBMT group with *Helicobacter pylori* status results after eradication comprised the study subjects and were included in the PP analysis.

**Helicobacter pylori eradication rates**

The *H. pylori* eradication rates at the end of the follow-up phase were 16.0% (4/25) in the PBAT group and 65.5% (19/29) in the PBMT group by ITT analysis (Table 2). Based on PP analysis, *H. pylori* eradication was achieved in 17.4% (4/23) of the PBAT group and in 70.4% (19/27) in the PBMT group. *Helicobacter pylori* eradication rates in the PBAT group were significantly lower than in the PBMT group by both ITT and PP analyses (*P* < 0.001, and *P* < 0.001).

**Adverse events and drug compliance**

Adverse events were reported in 7/25 patients (28.0%) in the PBAT group and in 5/29 patients (17.2%) in the PBMT group. Adverse event rates were similar in the two groups (P = 0.343); reported side-effects are shown in Table 3. In addition, drug compliance was similar in the two groups (Table 1). Two patients dropped out of each group due to adverse drug events (one patient

**Antimicrobial resistance of* Helicobacter pylori* and the outcome of eradication**

Thirty-one *H. pylori* isolates (17 in the PBAT group and 14 in the PBMT group) were collected before these quadruple treatments and submitted for antimicrobial sensitivity tests (Table 1). Resistance to amoxicillin or metronidazole was found in seven (22.6%) and 20 strains (64.5%), respectively. No strains developed resistance to tetracycline. Combined resistance to amoxicillin and metronidazole was found in four patients (12.9%).

The PP eradication rates of *H. pylori* isolates with amoxicillin resistance was 0% (0/4) and 16.7% (2/12) with amoxicillin–susceptible *H. pylori*, which was not significantly different to that in the PBAT group (*P* = 1.000; Fig. 1). Although all *H. pylori* isolates were found to be susceptible to tetracycline, only two of 12 strains susceptible to both amoxicillin and tetracycline were eradicated. In the PBMT group, those infected with metronidazole-susceptible *H. pylori* isolates achieved a higher PP eradication rate than those infected with metronidazole-resistant *H. pylori* isolates, but study statistical power was too low to determine the significance (83.3% vs 28.6%, *P* = 0.103).

**Table 1** Baseline patient demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>PBAT group (%)</th>
<th>PBMT group (%)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Included in ITT analysis</td>
<td>25</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>15/10</td>
<td>16/13</td>
<td>0.721†</td>
</tr>
<tr>
<td>Mean age ± SD (years)</td>
<td>58.6 ± 10.1</td>
<td>54.7 ± 12.3</td>
<td>0.213†</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>17</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Gastroduodenal ulcer</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Discontinued treatment</td>
<td>2</td>
<td>2</td>
<td>1.000†</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> isolates†</td>
<td>17 (68.0)</td>
<td>14 (48.3)</td>
<td>0.144†</td>
</tr>
<tr>
<td>AR</td>
<td>4 (23.5)</td>
<td>3 (21.4)</td>
<td>1.000†</td>
</tr>
<tr>
<td>MR</td>
<td>12 (70.6)</td>
<td>8 (57.1)</td>
<td>0.477†</td>
</tr>
<tr>
<td>AR + MR</td>
<td>2 (11.8)</td>
<td>2 (14.3)</td>
<td>1.000†</td>
</tr>
</tbody>
</table>

AR, amoxicillin resistance; MR, metronidazole resistance; PBAT, pantoprazole + bismuth + amoxicillin–clavulanate + amoxicillin; PBMT, pantoprazole + bismuth + metronidazole + tetracycline; ITT, intention-to-treat.

†All *H. pylori* isolates were susceptible to tetracycline.

**Table 2** *Helicobacter pylori* eradication rate

<table>
<thead>
<tr>
<th></th>
<th>PBAT % (n)</th>
<th>PBMT % (n)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eradication rate</td>
<td>16.0 (4/25)</td>
<td>65.5 (19/29)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.6–30.4</td>
<td>48.2–82.8</td>
<td></td>
</tr>
<tr>
<td>PP analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eradication rate</td>
<td>17.4 (4/23)</td>
<td>70.4 (19/27)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.9–32.9</td>
<td>53.2–87.6</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; ITT, intention-to-treat; PBAT, pantoprazole + bismuth + amoxicillin–clavulanate + amoxicillin; PBMT, pantoprazole + bismuth + metronidazole + tetracycline; PP, per-protocol.

†Two-sided Pearson’s χ² test.

**Table 3** Side-effects of quadruple therapy in *Helicobacter pylori*-infected patients

<table>
<thead>
<tr>
<th>Side-effects (n)</th>
<th>PBAT group (n = 25)</th>
<th>PBMT group (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea/vomiting</td>
<td>2 (8.0)</td>
<td>3 (10.3)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (16.0)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Constipation</td>
<td>—</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Epigastric pain</td>
<td>1 (4.0)</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>7 (28.0)</td>
<td>5 (17.2)</td>
</tr>
</tbody>
</table>

PBAT, pantoprazole + bismuth + amoxicillin–clavulanate + amoxicillin; PBMT, pantoprazole + bismuth + metronidazole + tetracycline.

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[in the PBAT group] due to epigastric pain and the other three patients due to nausea or vomiting).

Discussion

Quadruple therapy containing proton pump inhibitor, bismuth, tetracycline, and metronidazole has been generally used as the optimal second-line therapy after initial eradication failure, and is the regimen recommended by several guidelines. However, this regimen has been associated with eradication failure in more than 20% of cases by PP analysis. The main causes of failure are believed to be poor patient compliance and bacterial resistance. Therefore, many alternative therapeutic options with favorable compliance and various eradication rates have been suggested, but none of those achieves a 100% cure rate.

We hypothesized that reduced antimicrobial resistance due to the elimination of metronidazole from the quadruple regimen, and by replacing it with amoxicillin–clavulanate, which has high in vitro susceptibility, would increase H. pylori infection cure rates during second-line therapy. However, the results of our study show that the amoxicillin–clavulanate and tetracycline combination cannot be used to treat H. pylori infection, because it failed to eradicate infection in more than 80% of patients despite its high in vitro susceptibility. In fact, it eradicated H. pylori in only two of 12 patients who were infected with both amoxicillin- and tetracycline-susceptible H. pylori stains.

It is unclear why the amoxicillin–clavulanate–tetracycline combination performed so badly. A possible explanation for this finding is that the amoxicillin–clavulanate and tetracycline combination acted in an antagonistic manner. Results of the present study concur with those of an earlier study in which a regimen containing the amoxicillin and tetracycline/doxycycline combination eradicated H. pylori in only 20–35% of patients despite optimal patient compliance; however, antimicrobial susceptibility test results were not presented. It was suggested in that study that the bacterioidal activity of amoxicillin might have been hampered by the bacteriostatic effect of tetracycline, that is, via the inhibition of penicillin-binding protein synthesis, which is the main target of amoxicillin, and that the two antibiotics in the gastric lumen could interfere with the topical action of each other. Another ancillary study indirectly supports our results. It showed that a combination of tetracycline and amoxicillin failed to eradicate H. pylori in 33.3% (4/12) of patients despite 14-day treatments. All H. pylori strains isolated from the patients in that study were susceptible to metronidazole determined by Epsilometer-test, but without data on tetracycline. However, the combination of these two antibiotics without PPI may have altered in vivo antimicrobial effect; alternatively, although these antibiotics have excellent antimicrobial effects in organs such as the salpinx, they may not be as effective in the gastric lumen. Surprisingly, few data are available concerning the in vivo efficacy of the antimicrobial combination against microbial organisms, including H. pylori.

In contrast to our study, investigations in Taiwan and Italy using the amoxicillin and tetracycline–doxycycline combination reported favorable eradication rates, that is, 78%/89% and 91%/92%, by ITT/PP analysis, respectively. Moreover, the doses of tetracycline and amoxicillin or amoxicillin–clavulanate used by these various studies are similar, yet results are inconsistent. These inconsistencies do not allow us to arrive at a meaningful interpretation. However, we are of the opinion that use of the amoxicillin/amoxicillin–clavulanate and tetracycline/doxycycline antimicrobial combination be suspended for H. pylori eradication until its efficacy is understood. Moreover, the associations between the constituents of this combination in terms of their in vitro and in vivo effects require further investigation, which may explain why eradication rates do not reach 100%, even when the susceptibility of H. pylori is known.

Another important finding of the present study is that the eradication rate of standard second-line therapy was particularly poor with respect to metronidazole-resistant H. pylori infection. This result implies that metronidazole resistance has a negative impact on quadruple therapy containing metronidazole. In view of the high metronidazole resistance of H. pylori reported worldwide, this result indicates that metronidazole-containing quadruple therapies have limited use in second-line therapy. Therefore, it appears reasonable to try to trade off metronidazole for other...
antibiotics with known low resistance rates. However, it remains uncertain as to which antimicrobials should be included instead of metronidazole in quadruple regimens. Moreover, in the present study none of the six H. pylori strains resistant to amoxicillin was eradicated using the PBAT regimen, though two of 12 strains susceptible to amoxicillin were eradicated, but without a significant difference between the two. These findings reaffirm the importance of H. pylori resistance to antibiotics on H. pylori eradication rates. However, in the case of metronidazole- and amoxicillin-containing triple regimen as first-line or second-line therapy, metronidazole resistance could be overcome in some reports, with favorable eradication rates ranging from 70.0% to 81.8%.33,34 Therefore, metronidazole with these antimicrobial combinations could still be considered for H. pylori eradication even in areas with high prevalence of metronidazole-resistant H. pylori.

In conclusion, although the amoxicillin-clavulanate-tetracycline combination is seemingly attractive because of its high in vitro susceptibility, we cannot recommend it for H. pylori eradication.

Acknowledgments

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References


