RESISTANCE TO ANTIMICROBIAL AGENTS IN ERADICATION OF
HELICOBACTER PYLORI INFECTION

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Key Words: Amoxicillin, clarithromycin, drug resistance, eradication, Helicobacter pylori,
metronidazole, proton pump inhibitor, triple therapy.

Synopsis
With the recent increasingly widespread use of triple therapy to eradicate Helicobacter pylori (H. pylori) consisting of a proton pump inhibitor (PPI), amoxicillin (AMPC), and clarithromycin (CAM) [PPI/AC], the issue of resistance to antimicrobial agents has risen. In this study, we investigated the rate of increase in antimicrobial
resistance after eradication treatment and the mechanism of the increase in CAM resistance.

Subjects and Methods: The subjects were 277 patients with digestive diseases, all of whom were positive for *H. pylori*. Eradication therapy with PPI/AC was administered. Before and after the treatment, endoscopy was performed, and the gastric mucosal specimens were obtained from 2 sites, antrum and body. The presence or absence of *H. pylori* infection was evaluated by culture/histological examination. In all patients with positive reactions on culture, the susceptibility to AMPC, CAM, and metronidazole (MNZ) was measured by the agar plate dilution method.

Results: The rate of eradication was 83.7% (216/258, per protocol). Prior to the treatment, the rates of bacteria resistant strains to AMPC, CAM, or MNZ were 1.1%, 6.9%, and 2.6%, respectively. The susceptibility to AMPC/CAM in both the antrum and body regions could be measured in 212 and 208 patients, respectively. Prior to the treatment, 1.4% (3/212) and 8.6% (18/208) of the patients were resistant to AMPC and CAM, respectively. After the treatment, the percentages were increased to 5.4% (2/37) and 64.9% (24/37), respectively. In addition, concerning CAM, in 5 patients, CAM-sensitive bacteria were detected in one of the two regions, the antrum and body, while CAM-resistant bacteria were detected in the other region (mixture of resistant and sensitive bacteria). In these patients, eradication was all unsuccessful. After the treatment, CAM-resistant bacteria were detected in both the antrum and body.

Conclusions: The rate of eradication with PPI/AC therapy was 80% or more; however, the rate of CAM-resistant bacteria was increased after the treatment. These results suggest that the acquisition of CAM resistance by *H. pylori* and bacterial selection of *H. pylori* are involved in the increase after eradication treatment.

Introduction

In November 2000, *Helicobacter pylori* (*H. pylori*) eradication therapy for peptic ulcers was approved for reimbursement by the national health insurance of Japan. The eradication regimen consists of triple therapy with a proton pump inhibitor (PPI), amoxicillin (AMPC), and clarithromycin (CAM) [PPI/AC]. In Japan, the rate of eradication by triple therapy ranges from 80% to 95% (Miwa, Ohkura et al., 1999). Many studies have reported that there are no significant differences in the rate of eradication when any of the 3 agents, omeprazole (OPZ), lansoprazole (LPZ), and rabeprazole (RPZ),
is used as a PPI (Miwa, Ohkura et al., 1999; Inaba, Mizuno et al., 2002) however, Murakami et al., (2002) reported that RPZ achieved a higher rate of eradication than LPZ. In addition, several studies have indicated that there are no significant differences in the rate of eradication among different doses of RPZ as a PPI, 10 mg, 20 mg, and 40 mg (Hokari, Sugiyama et al., 2001; Miwa, Yamada et al., 2000). Similarly, there were no significant differences in the rate of eradication between 2 doses of CAM: 400 mg and 800 mg (Murakami, Sato et al., 2002; Hokari, Sugiyama et al., 2001; Kihira, Satoh et al., 2000).

It has been reported that factors involved in failure in eradication by PPI/AC therapy include compliance, age, smoking, CYP2C19 gene polymorphism, resistance to antimicrobial agents, and values of urea breath test before the treatment (Hokari, Sugiyama et al., 2001; Perri, Villani et al., 2001; Kamada, Haruma et al., 1999; Furuta, Naohito et al., 2001; Poon, Chang et al., 2002). Miwa, Misawa et al., (2001) indicated that among these factors, CAM resistance most markedly influenced success in eradication.

In this study, we investigated the rate of increase in antimicrobial resistance after eradication treatment of PPI/AC therapy and the mechanism of the increase in CAM resistance.

**Subjects and Methods**

**Subjects:** The subjects were 277 patients who underwent upper gastrointestinal endoscopy between April 1995 and October 1999, and were positive for *H. pylori* on culture of the gastric mucosa. These patients consisted of 197 males and 80 females, with a mean age of 49.4±12.7 years. Diseases consisted of gastric ulcer in 95 patients, duodenal ulcer in 85 patients, gastric and duodenal ulcers in 51 patients, and chronic gastritis in 46 patients. Approval for this study was granted by the hospital clinical ethics committee, and fully informed consent was obtained from all subjects prior to participation.

**Eradication method:** After informed consent was obtained from all subjects, 6 regimens were administered as triple therapy. In the OAC group, 20 mg/day of OPZ as a PPI, 1,500
mg/day of AMPC, and 600 mg/day of CAM were administered for 1 week. In the OAC2 group, the dose of OPZ was increased to 40 mg/day, and the above doses of AMPC and CAM were administered for 1 week. In the OACE group, OAC and a mucosal protective factor preparation, Ecabet sodium (3.0 g/day), were simultaneously administered for 1 week. In the OACR group, similarly, OAC and a mucosal protective factor preparation, rebamipide (300 mg/day), were simultaneously administered for 1 week. In the LAC group, 30 mg/day of LPZ as a PPI as well as AMPC and CAM at the doses described in the OAC group were administered for 1 week. In the LACE group, 60 mg/day of LPZ as a PPI, AMPC and CAM at the doses described in the OAC group, and 3.0 g/day of Ecabet sodium were administered for 1 week.

*Isolation and culture of H. pylori:* Biopsy specimens of the gastric mucosa were obtained by endoscopy from the greater curvature of the antrum and body of the stomach, homogenized, inoculated to Skirrow agar and cultured under microaerophilic conditions (O2: 5%, CO2: 10%, N2: 85%) at 37°C for 5 to 7 days.

*Measurement of the minimum inhibitory concentration (MIC):* In accordance with the standard procedure established by the Japanese Society of Chemotherapy (1981), MIC was measured by the agar plate dilution method. One colony was collected from Skirrow agar modified, and cultured in sheep blood agar medium for 48 hours. Then, for preculture, bacteria were inoculated to Brucella Broth containing fetal bovine serum (FBS, JRH Biosciences, Kansas, and U.S.A) at 5% (v/v), and shaking culture was performed overnight. To measure MIC, bacterial solution was inoculated using a microplanter (Sakuma Seisakusho, Tokyo, Japan) in which agents were added to MH agar containing equine defibrinated blood at 5% (v/v), and MIC was evaluated 48 hours after inoculation. As antimicrobial agents, the MICs of AMPC, CAM, and metronidazole (MNZ) were measured. Strains with MIC values of 0.5 μg/ml or more, 1 μg/ml or more, and 12.5 μg/ml or more were regarded as resistant to AMPC, CAM, and MNZ, respectively.
Histological examination of *H. pylori*: The biopsy specimens of the gastric mucosa obtained endoscopically from the greater curvature of antrum and body by endoscopy were fixed in formalin for hematoxylin and eosin/Giemsa staining. Light microscopy was performed to investigate the presence or absence of *H. pylori*.

Evaluation of eradication: Four weeks after the end of eradication therapy, upper gastrointestinal endoscopy was performed. Biopsies were obtained from the greater curvature of antrum and body (2 areas per greater curvature). Patients negative on both culture and histological procedures were regarded as exhibiting successful eradication. The MICs of AMPC and CAM were measured in patients with positive reactions on culture after the treatment.

Results

Results of eradication: Overall, eradication was successful in 216 patients, but unsuccessful in 42 patients. Nineteen patients dropped out of this study. The rates of eradication were 83.7% (216/258) per protocol and 78.0% (216/277) in patients with intention to treat. Among the eradication regimens, there were no significant differences in the eradication rate of each regimen, as shown in Table I.

MICs of various antimicrobial agents:

1) Prior to eradication treatment. Prior to eradication therapy, the MICs of AMPC, CAM, and MNZ could be measured in 456, 452, and 446 strains of *H. pylori*, respectively. Concerning AMPC, 265 strains showed an MIC value of 0.0125 μg/ml, which was the highest percentage (58.1%), and 5 strains (1.1%) were resistant to AMPC, with an MIC value of 0.5 μg/ml or more. Regarding of CAM, 157 strains showed an MIC value of 0.05 μg/ml, which was the highest percentage (34.7%), and 31 strains (6.9%) were resistant to CAM, with an MIC value of 1 μg/ml or more. Concerning MNZ, 195 strains showed an MIC value of 3.13 μg/ml, which was the highest percentage (43.7%), 12 strains (2.6%) were resistant to MNZ, with an MIC value of 12.5 μg/ml or more (Table II).
### TABLE I
Eradication rate with respect to eradication regimens

<table>
<thead>
<tr>
<th></th>
<th>LACE</th>
<th>OAC</th>
<th>OAC2</th>
<th>OACE</th>
<th>OACR</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Success</td>
<td>22</td>
<td>43</td>
<td>36</td>
<td>19</td>
<td>40</td>
<td>216</td>
</tr>
<tr>
<td>Failure</td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>Dropout</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>Cases</td>
<td>30</td>
<td>55</td>
<td>44</td>
<td>24</td>
<td>55</td>
<td>277</td>
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</table>

_Cure Rate %_

<table>
<thead>
<tr>
<th></th>
<th>ITT</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>73.3</td>
<td>84.6</td>
</tr>
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</table>

LAC: lansoprazole 30mg/day + Amoxicillin 1500mg/day + clarithromycin 600mg/day
LACE: lansoprazole 30mg/day + Amoxicillin 1500mg/day + clarithromycin 600mg/day + Ecabetsodium 3000mg/day
OAC: omeprazole 20mg/day + Amoxicillin 1500mg/day + clarithromycin 600mg/day
OAC2: omeprazole 40mg/day + Amoxicillin 1500mg/day + clarithromycin 600mg/day
OACE: omeprazole 20mg/day + Amoxicillin 1500mg/day + clarithromycin 600mg/day + Ecabet sodium 3000mg/day
OACR: omeprazole 20mg/day + Amoxicillin 1500mg/day + clarithromycin 600mg/day + rebamipide 300mg/day
ITT: Intention to Treat; PP: Per Protocol

### TABLE II
MIC's of various antimicrobial agents for _H. pylori_ prior to eradication

<table>
<thead>
<tr>
<th>MIC µg/ml</th>
<th>AMPC</th>
<th>%</th>
<th>CAM</th>
<th>%</th>
<th>MNZ</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>0.0125</td>
<td>265</td>
<td>58.1</td>
<td>85</td>
<td>18.8</td>
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<tr>
<td>0.025</td>
<td>83</td>
<td>18.2</td>
<td>119</td>
<td>26.3</td>
<td>0</td>
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</tr>
<tr>
<td>0.05</td>
<td>57</td>
<td>12.5</td>
<td>157</td>
<td>34.7</td>
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<td>0.0</td>
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<tr>
<td>0.1</td>
<td>32</td>
<td>7.0</td>
<td>45</td>
<td>10.0</td>
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<td>0.2</td>
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<td>0.2</td>
<td>8</td>
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<td>8</td>
<td>1.8</td>
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<tr>
<td>0.39</td>
<td>6</td>
<td>1.3</td>
<td>4</td>
<td>0.9</td>
<td>6</td>
<td>1.3</td>
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<tr>
<td>0.78</td>
<td>4</td>
<td>0.9</td>
<td>3</td>
<td>0.7</td>
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<td>1.56</td>
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<tr>
<td>3.13</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
<td>0.4</td>
<td>195</td>
<td>43.7</td>
</tr>
<tr>
<td>6.25</td>
<td>0</td>
<td>0.0</td>
<td>4</td>
<td>0.9</td>
<td>57</td>
<td>12.8</td>
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<tr>
<td>12.5</td>
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<td>0.2</td>
<td>1</td>
<td>0.2</td>
<td>10</td>
<td>2.2</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>0.0</td>
<td>16</td>
<td>3.5</td>
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<td>0.0</td>
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<tr>
<td>50</td>
<td>0</td>
<td>0.0</td>
<td>8</td>
<td>1.8</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>456</td>
<td></td>
<td>452</td>
<td></td>
<td>446</td>
<td></td>
</tr>
</tbody>
</table>

AMPC: amoxicillin, CAM: clarithromycin, MNZ: metronidazole
**TABLE III**

Eradication rate with respect to CAM susceptibility prior to eradication

<table>
<thead>
<tr>
<th>Eradication Rate</th>
<th>CAM Sensitive</th>
<th>CAM Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate</td>
<td>88.4% (168/190)</td>
<td>11.1% (2/18)</td>
</tr>
</tbody>
</table>

**TABLE IV**

Changes in CAM susceptibility after eradication of *H. pylori* with respect to gastric sites

<table>
<thead>
<tr>
<th>Type</th>
<th>Before</th>
<th>After</th>
<th>n</th>
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<tr>
<td>I</td>
<td>S-S</td>
<td>S-S</td>
<td>13</td>
</tr>
<tr>
<td>II</td>
<td>S-S</td>
<td>R-R</td>
<td>9</td>
</tr>
<tr>
<td>III</td>
<td>S-R</td>
<td>R-R</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>R-R</td>
<td>R-R</td>
<td>10</td>
</tr>
</tbody>
</table>

S = Sensitive strain; R = Resistant strain

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**Figure 1**

Sensitive strains → Mixture of sensitive and resistant strains (appearance of resistant strains)

- Point mutation (use of antimicrobial agents in other diseases)
- Resistant strains
- Bacterial selection (eradication)
- Point mutation (eradication)
The MIC of AMPC was measured in both the antrum and body in 212 patients. Of these patients, 209 patients were sensitive to AMPC, while 3 patients (1.4%) were resistant to AMPC prior to the treatment. The MIC of CAM was measured in both of the two regions in 208 patients of whom 190 were sensitive to CAM, while 18 (8.6%) were resistant to CAM prior to the treatment.

2) Treatment-related changes in drug susceptibility and eradication rates with respect to susceptibility. Of three AMPC-resistant patients, eradication was successful in 1 patient, while the results were unsuccessful in 2 patients. The 2 patients with unsuccessful treatment were resistant to both AMPC and CAM. There was no acquisition of resistance in the AMPC-sensitive patients. Of 18 CAM-resistant patients, eradication was successful in 2 patients, but unsuccessful in 16 patients. Eradication was successful in 168 patients of 190 CAM-sensitive patients, but unsuccessful in 22 patients. Thirteen of the 22 patients remained sensitive to CAM, while 9 patients acquired resistance. The rates of eradication were 88.4% (168/190) in CAM-sensitive patients and 11.1% (2/18) in CAM-resistant patients (Table III). MIC values were measured in both the antrum and body in 37 of 42 patients with unsuccessful treatment. After the treatment, 2 patients (5.4%) were resistant to AMPC, and 24 patients (64.9%) were resistant to CAM.

3) Changes in CAM susceptibility after the treatment. In 37 patients with the unsuccessful eradication in whom MIC values could be measured in both the antrum and body before and after the treatment, we summarized changes in CAM susceptibility with respect to gastric sites. As shown in Table IV, the changes were classified into 4 patterns. In type I, CAM-sensitive bacteria were detected in both the antrum and body before and after the treatment. In type II, CAM-sensitive bacteria were detected in both the antrum and body before the treatment, but CAM-resistant bacteria were detected in both regions after the treatment. In type III, before the treatment CAM-sensitive bacteria were detected in one regions and CAM-resistant bacteria in the other region; however, after the treatment,
CAM-resistant bacteria were detected in both the two regions. In type IV, CAM-resistant bacteria were detected in both the antrum and body before and after the treatment. In this study, type I changes in CAM susceptibility were evaluated as in 13 patients, type II in 9 patients, type III in 5 patients, and type IV in 10 patients (Table IV).

Discussion

Triple therapy with PPI/AC achieves eradication of *H. pylori* infection in 80 to 90% of patients, with a low incidence of side effects (Asaka, Satoh *et al.*, 2001). This therapy is routinely administered in Japan. However, some problems have risen with the widespread use of this eradication regimen. One issue is drug resistance, especially CAM resistance. When the results of PPI/AC therapy are unsuccessful, the proportion of CAM-resistant patients increases after the treatment. Murakami, Sato *et al.*, (2001) and Tankovic, Lamarque *et al.*, (2001) reported percentages of CAM-resistant patients were 66.2% and 70%, respectively. The other issue is that PPI/AC therapy is only approved for reimbursement by the national health insurance in Japan as a secondary eradication regimen. When the same regimen (PPI/AC therapy) is additionally used for secondary eradication therapy in patients in whom PPI/AC therapy results in unsuccessful outcome, and the rate of eradication is extremely low. Nagahara, Miwa *et al.*, (2001) reported that the rate of eradication was 52.9%. In this study, we investigated the susceptibility change of *H. pylori* to various antimicrobial agents and the role of CAM resistance in triple therapy with PPI/AC.

In an AMPC susceptibility test, the proportion of resistant bacteria was extremely low, being 1.1% before treatment. Aldana, Kato *et al.*, (2002) and Murakami, Fujioka *et al.*, (2002) reported that the rates of AMPC-resistant bacteria were 0.3% and 0.2%, respectively in Japan. A study of drug sensitivity in Europe also reported that the proportion of AMPC-resistant bacteria was 0.9% (Boyanova, Mentis *et al*; 2002). However in Brazil, the proportion of AMPC-resistant bacteria was 29% (Mendonca,
Ecclissato et al.; 2000,). Furthermore, there was no PPI/AC therapy-related acquisition of AMPC resistance in this study. Previous studies have not demonstrated PPI/AC therapy-related acquisition of AMPC resistance (Murakami, Sato et al., 2001, Adamek, Suerbaum et al., 1998). It has been reported that the mechanism of AMPC resistance is associated with changes in penicillin-binding proteins, not with production of β lactamase (Dore, Graham et al., 1999, Okamoto, Yoshiyama et al., 2002).

The proportion of MNZ-resistant bacteria was 2.6% prior to eradication. In Japan, Aldana, Kato et al., (2002) and Murakami, Fujioka et al., (2002) reported that the percentages were 9% and 26.7%, respectively, showing a large deviation in data between the hospitals. However, Murakami, Fujioka et al., (2002) used epsilometer test (E test) as the drug susceptibility test. According to Glupcznski, Broutet et al., (2002) concerning AMPC and CAM, there was an excellent correlation between the results of the E test and the agar plate dilution method. However, concerning MNZ, the E test revealed higher MIC values (>2 log₂). In Europe and Brazil, the proportions of MNZ-resistant bacteria were 37.9% and 42%, respectively, being higher than in Japan. It has been reported that rdxA is the main factor involved in the mechanism of MNZ resistance (Goodwin, Kersulyte et al., 1998; Jeong, Mukhopadhyay, et al., 2001).

Prior to eradication treatment, the percentage of CAM-resistant bacteria was 6.9%. Aldana, Kato et al., (2002) and Murakami, Fujioka et al., (2002) reported that percentages of CAM-resistant bacteria were 11.0% and 12.9%, respectively. In our hospital, the percentage was much lower. In Europe and Brazil, percentages were 9.5% and 7%, respectively. In the mechanism of CAM resistance, Debets-Ossenkopp, Sparrius et al., (1996) and Versalvoic, Shortrige et al., (1996) reported that point mutations of H. pylori 23SrRNA are etiologically involved. In this study, we further investigated changes in CAM susceptibility after the treatment in patients in whom PPI/AC therapy was unsuccessful. In an initial study of CAM resistance before eradication only CAM-resistant bacteria were detected in both the antrum and body in some patients, while both
CAM-resistant and CAM-sensitive bacteria were mixed in both regions in other patients. Maeda, Yoshida et al., (2000) also reported patients with a similar mixture of CAM-resistant and CAM-sensitive bacteria. In these patients, eradication was unsuccessful, and after the treatment, only CAM-resistant bacteria were detected in both the antrum and body. Therefore, acquisition of CAM resistance and bacterial selection by \textit{H. pylori} may have been involved in the increase in CAM-resistant bacteria after the treatment. Briefly, in patients in whom CAM-resistant and CAM-sensitive bacteria were mixed, PPI/AC therapy may have eradicated only CAM-sensitive bacteria, while only CAM-resistant bacteria may have survived (Figure 1). This may also be associated with the finding that PPI/AC therapy eradicated a high percentage of CAM-sensitive bacteria, but eradicated only 11.1% of CAM-resistant bacteria in this study.

CAM resistance may be the most important factor in PPI/AC therapy. Previous investigation of CAM resistance have performed endoscopy to collect the gastric mucosa, isolate and culture bacterial strains, and then a susceptibility test or polymerase chain reaction (PCR) have been carried out, as in this study. However, endoscopy is invasive, and new procedures without endoscopy have been developed. A procedure in which the presence or absence of CAM resistance is investigated using paraffin embedded gastric biopsy specimens (Russman, Schmidt et al., 2003) and a procedure in which CAM resistance is investigated in stool \textit{H. pylori} (Fontana, Favaro et al., 2003) have been reported. In the near future, we hope to develop eradication therapy appropriate for each individual patient after investigating CAM susceptibility with these new procedures.

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References


