### Helicobacter pylori Resistance to Rifabutin in the Last 7 Years

Toshihiro Nishizawa, Hidekazu Suzuki, Juntao Matsuzaki, Hiroe Muraoka, Hitoshi Tsugawa, Kenro Hirata, and Toshifumi Hibi

Division of Gastroenterology, National Hospital Organization Tokyo Medical Center, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, and Mitsubishi Chemical Medience Corporation, Tokyo, Japan

Received 1 August 2011/Returned for modification 22 August 2011/Accepted 24 August 2011

A low rate of resistance (0.24%) to rifabutin was noted in Helicobacter pylori strains isolated from 414 Japanese patients. The only rifabutin-resistant strain detected showed a point mutation in the rpoB gene and was isolated from a patient with a history of rifampin treatment for pulmonary tuberculosis.

Resistance to antibiotics is a major cause of failure of Helicobacter pylori eradication therapy (4, 5, 8), and alternative regimens need to be developed to overcome this problem (6). One of the candidate antibiotics for a third-line eradication regimen is a fluoroquinolone, such as levofloxacain or sitafloxacin (13). We previously examined the resistance of H. pylori to gatifloxacin (8-methoxy fluoroquinolone) and showed high rates of resistance (43%) to gatifloxacin of H. pylori strains isolated from Japanese patients after unsuccessful eradication therapy (10).

Another candidate antibiotic for a third-line eradication regimen is rifabutin, which is an antituberculous agent derived from rifamycin-S, which is structurally similar to rifampin. Rifabutin inhibits the beta-subunit of DNA-dependent RNA polymerase of H. pylori, which is encoded by the rpoB gene (2). Van der Poorten and Katelaris designed a prospective study to assess the efficacy of rifabutin-based triple therapy. Of 67 patients, eradication of H. pylori was achieved in 76% (48/63) in the per protocol analysis and 72% (48/67) in the intention-to-treat analysis (15). Adverse events were reported in 10% of the patients. This study demonstrated that rifabutin triple therapy as third-line therapy is well tolerated and yields an acceptable eradication rate.

We previously investigated the MICs of rifabutin and also the point mutations of the rpoB gene in 48 strains of H. pylori isolated from patients of a general hospital (Keio University Hospital) and 46 strains isolated from patients at a specialized hospital for chronic respiratory diseases (Minami-Yokohama Hospital) (14). Although all the strains isolated from the patients at the general hospital were susceptible to rifabutin, 8 of the 46 strains (17.4%) isolated from the patients at the specialized hospital for chronic respiratory diseases showed resistance to rifabutin; 6 of these 8 strains also showed point mutations of the rpoB gene. In particular, the MICs were high for the strains isolated from patients with a history of treatment with rifampin. The present study was aimed at analyzing the resistance pattern to rifabutin of H. pylori strains isolated from the patients at the general hospital (Keio University Hospital) during the last 7 years.

The present study was conducted with the approval of the Ethics Committee of Keio University School of Medicine. A total of 414 patients with H. pylori infection (233 males and 181 females; mean age, 55.9 years) were enrolled between September 2004 and July 2011. H. pylori infection was defined as H. pylori culture positivity in this study. All patients underwent esophagogastroduodenoscopy and gastric biopsy for bacterial culture at Keio University Hospital. Of the total, 19 patients had no history of H. pylori eradication therapy, 263 patients had had one treatment failure, 116 patients had had two treatment failures, and 16 patients had had three treatment failures (first-line treatment was triple therapy with 800 mg/day clarithromycin, 1,500 mg/day amoxicillin, and a proton pump inhibitor [PPI] for 7 days; second-line treatment was triple therapy with 500 mg/day metronidazole, 1,500 mg/day amoxicillin, and a PPI for 7 days; and third-line treatment was triple therapy with a fluoroquinolone [400 mg/day levofloxacin, gatifloxacin, or sitafloxacin], 2,000 mg/day amoxicillin, and a PPI for 7 days) (3, 9, 11). Among the 386 patients, the 48 strains isolated between September 2004 and June 2005 that were used in the present study had been previously examined, and a comparison of the rifabutin resistance rates between isolates from the general hospital patients and patients at the specialized hospital for chronic respiratory diseases was reported (14).

The susceptibility of H. pylori isolates to rifabutin was determined by the agar dilution method, according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (1, 7). Isolates were considered resistant to rifabutin if the MIC of the drug was ≥0.25 μg/ml (14). The MIC of rifabutin for one of the 414 (0.24%) strains was high (MIC = 2 μg/ml), whereas the MIC values for the other strains were low (MIC < 0.015 μg/ml). The rifabutin-resistant strain was from a patient who had a history of one H. pylori eradication failure and a history of treatment with rifampin for pulmonary tuberculosis.

We performed PCR amplification and sequencing of the resistance-determining regions of the rpoB gene (from codon 511 to 612; forward, 5'-AAATGATCACAAGCACCATC-3' and reverse, 5'-ACCTTGCCATCCACAACC-3') (2) of 43 strains isolated between June 2008 and May 2010. The rpoB sequences obtained were compared with the published rpoB sequences obtained were compared with the published rpoB sequences.
testing prior to the inclusion of rifabutin in treatment regimens should be given to the results of though rifabutin may be a promising candidate for third-line multiresistant that the use of rifabutin be reserved for the treatment of rifabutin has fortunately remained low. It has been suggested that rifabutin-susceptible strains (12). Al-

### Table 1. MIC of rifabutin and substitution in the resistance-determining region of the \( rpoB \) gene

<table>
<thead>
<tr>
<th>Strain(s)</th>
<th>Rifabutin MIC (( \mu )g/ml)</th>
<th>Substitution in the ( rpoB ) gene</th>
<th>History of treatment with rifabutin</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSO513</td>
<td>2</td>
<td>D530N</td>
<td>Yes</td>
</tr>
<tr>
<td>42 other strains</td>
<td>&lt;0.015</td>
<td>Not detected</td>
<td>No</td>
</tr>
</tbody>
</table>

data of rifabutin-susceptible \( H. pylori \) strain 26695 (GenBank accession number AE000511). The rifabutin-resistant strain had a point mutation of the \( rpoB \) gene with a substitution at amino acid 530 (D530N). On the other hand, no such mutations were seen in the remaining 42 rifabutin-susceptible strains (Table 1).

In conclusion, the prevalence of resistance of \( H. pylori \) to rifabutin has fortunately remained low. It has been suggested that the use of rifabutin be reserved for the treatment of multiresistant \( Mycobacterium tuberculosis \) strains (12). Although rifabutin may be a promising candidate for third-line therapy, careful consideration should be given to the results of the history of treatment with rifampin or of drug susceptibility testing prior to the inclusion of rifabutin in treatment regimens for eradication of \( H. pylori \).

The present study was supported by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the promotion of Science (No. 22300169, to H.S.), a grant of the Adaptable and Seamless Technology Transfer Program through target-driven R&D (A-STEP) (AS231Z00132G to H.S.) from the Japan Science and Technology Agency (JST), and grants from the Smoking Research Foundation (B) from the Japan Society for the promotion of Science. The present study was supported by a Grant-in-Aid for Scientific Research (C) (to H.S.) from the Japan Society for the promotion of Science (JSPS) (KAKENHI No. 25860695, to H.S.), a grant of the Nateglinide Memorial Toyoshima Research and Education Fund (to H.S.), the Keio Gijuku Academic Development Fund (to H.S.), and grants from the Smoking Research Foundation (to H.S.).

### REFERENCES