Influence of CYP2C19 Polymorphism and *Helicobacter pylori* Genotype Determined From Gastric Tissue Samples on Response to Triple Therapy for *H pylori* Infection

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Background & Aims: **The relationship between single nucleotide polymorphisms (SNPs) and clinical outcomes has been intensively studied. We intended to determine SNPs of CYP2C19 and 23S rRNA of *Helicobacter pylori* by using rapid urease test (RUT)–positive gastric mucosal samples.** ***Methods:*** **One hundred thirty-nine patients with *H pylori*–positive results based on RUT completed 1-week treatment with lansoprazole 30 mg twice a day, clarithromycin 200 mg 3 times daily, and amoxicillin 500 mg 3 times daily. SNPs from adenine to guanine at positions 2142 and 2143 of 23S rRNA of *H pylori* (A2142G and A2143G) and SNPs from guanine to adenine at positions 681 in exon 5 (**2*) and 636 in exon 4 (**3*) of CYP2C19 were determined by the serial invasive signal amplification reaction assay by using DNAs extracted from gastric tissue samples already used for RUT. Minimum inhibitory concentrations of clarithromycin for *H pylori* were determined by culture test. CYP2C19 genotypes were classified into the rapid metabolizer (**1/*1*), intermediate metabolizer (**1/*2* or **1/*3*), and poor metabolizer (**2/*2*, **2/*3*, or **3/*3*) groups.** ***Results:*** ***H pylori*** **strains with A2142G or A2143G mutation had higher minimum inhibitory concentrations for clarithromycin. Cure rates in rapid, intermediate, and poor metabolizer groups were 57.8% (95% confidence interval, 42.1%–72.4%), 88.2% (78.1%–94.8%), and 92.3% (74.9%–99.1%), respectively ($P < .001$). Cure rates in strains with and without A2142G or A2143G mutation were 48.3% (29.4%–67.5%) and 87.3% (79.5%–92.7%), respectively ($P < .001$).** ***Conclusions:*** **SNPs of CYP2C19 and 23S rRNA of *H pylori* using RUT-positive gastric mucosal samples could be predictable determinants for *H pylori* eradication by triple therapy.**

Eradication of *Helicobacter pylori* infection is now performed for the treatment of upper gastrointestinal disorders, such as peptic ulcer diseases, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and after early resection of gastric cancer, as well as the extragastrointestinal disorders, such as idiopathic thrombocytopenia, iron deficiency anemia, and chronic urticaria.[1–7] Current treatment strategies for the eradication of *H pylori* include a proton pump inhibitor (PPI) and 1 or 2 antibacterial agents, such as amoxicillin, clarithromycin, and metronidazole.[8–11]

PPIs, such as lansoprazole and omeprazole, are mainly metabolized in the liver by a genetically determined enzyme, *S*-mephenytoin 4′-hydroxylase (CYP2C19).[12–19] We reported that plasma concentrations of PPIs and their effects on intragastric pH depended to a significant extent on the CYP2C19 genotype status.[20–23] Moreover, we reported that the CYP2C19 genotype status and bacterial susceptibility to clarithromycin were each significantly related to eradication rates of *H pylori* by the triple therapy with a PPI, clarithromycin, and amoxicillin.[24–26] Interestingly, genetic differences in CYP2C19 genotypes and bacterial susceptibility to clarithromycin are both due to the single nucleotide polymorphisms (SNPs) of the CYP2C19 gene and the 23S rRNA gene of *H pylori*, respectively.[27–34] Therefore, the development of an inexpensive and reliable high-throughput method for scoring of such SNPs is imperative

Abbreviations used in this paper: CI, confidence interval; ^{13}C-UBT, ^{13}C-urea breath test; CYP2C19, *S*-mephenytoin 4′-hydroxylase; DU, duodenal ulcer; GU, gastric ulcer; IM, intermediate metabolizer; MALT, mucosa-associated lymphoid tissue; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; PM, poor metabolizer; PPI, proton pump inhibitor; RFLP, restriction fragment length polymorphism; RM, rapid metabolizer; RUT, rapid urease test; wt, wild-type.

1542-3565/05/$30.00
PII: 10.1053/S1542-3565(04)00779-7

in the *H pylori* eradication therapy with a regimen including a PPI and clarithromycin.

The serial invasive signal amplification reaction assay is one of the diagnostic tools to determine SNPs with a high specificity and sensitivity from unamplified (or amplified) genomic or complementary DNA samples.[35,36] Its technology is easy, inexpensive, and amenable to automation, which can yield a production rate of thousands of assays per day.[37–40] The serial invasive signal amplification reaction assay system is now thought to be one of the clinically useful tools for the individualized medicine based on genetics.

In the present study, we developed the serial invasive signal amplification reaction assay system for the determination of SNPs of CYP2C19 (ie, **2* in exon 5 and **3* in exon 4) and 23S rRNA of *H pylori* (from adenine to guanine mutation at the positions of 2142 and 2143) from the genomic DNA samples extracted from gastric mucosal samples that had already been used for rapid urease test (RUT), and we examined whether the results obtained from these methods were related to the success or failure of eradication of *H pylori* by a triple therapy with lansoprazole, amoxicillin, and clarithromycin.

Methods

Patients

The study subjects consisted of 141 patients with gastric ulcer (GU, n = 34), duodenal ulcer (DU, n = 34), or gastritis (n = 73), (male/female = 122/19; median age [range], 51 y [17–78 y]; and body weight [range], 65.0 kg [45.0–94.7 kg]). These patients had endoscopically and histologically proven ulcer or active chronic gastritis and were all *H pylori*–positive on the basis of RUT as described later. Written informed consent for participation was obtained from each of the patients before the study when the positive RUT result was obtained. The study protocol was approved in advance by the Human Institutional Review Board of Hamamatsu University School of Medicine. Throughout the study period, the investigators involved in the assessment of eradication of *H pylori* were unaware of CYP2C19 genotype and clarithromycin-resistant test results.

Determination of *H pylori* Infection by Rapid Urease Test and Culture Test

During gastroduodenoscopy, several biopsy specimens from both the antrum and corpus of the greater curvature were obtained for RUT and bacteriologic culture. For RUT, the biopsy specimens were inoculated into the HelicoCheck (Otsuka Pharmaceutical Co, Ltd, Tokyo, Japan). A positive result was recorded when the color changed from yellow to pink within 24 hours. The positive RUT samples were stored at −20°C.

For bacterial culture and antimicrobial sensitivity testing, the biopsy samples were inoculated onto agar plates developed by Dent and McNulty[41] and were incubated at 37°C under microaerophilic conditions for up to 7 days. Colonies were identified as *H pylori* on the basis of morphology in Gram stains, oxidase and catalase tests, and RUT results.[42] When the culture test result for *H pylori* was positive, colonies were then subcultured to determine minimum inhibitory concentrations (MICs) of amoxicillin and clarithromycin by the agar dilution method. The cutoff concentrations used to define resistance were >0.5 μg/mL for amoxicillin and >1.0 μg/mL for clarithromycin.[43]

Treatment for *H pylori* Infection

For eradication of *H pylori*, 30 mg lansoprazole twice daily, 200 mg clarithromycin 3 times a day, and 500 mg amoxicillin 3 times a day were administered for 1 week. In addition, patients with GU or DU were treated with a daily dose of 30 mg lansoprazole for 5 to 7 weeks after the triple therapy. Endoscopic examination and determination of *H pylori* status were performed before and 1 month after the end of all treatments including PPI given alone.

One month after the treatment, all patients also underwent ^{13}C-urea breath test (^{13}C-UBT) as described in our previous report.[44] All ^{13}C-UBT results were calculated as delta per mil (Δ‰) increase in $^{13}CO_2/^{12}CO_2$ ratio at 20 minutes above the baseline value obtained before administration of 100 mg ^{13}C-urea. Values less than 2.5‰ were taken to indicate a lack of infection with *H pylori*.[44]

Eradication of *H pylori* was judged on the basis of the results of RUT and ^{13}C-UBT. When all of these tests yielded negative results 1 month after treatment, eradication of *H pylori* was judged to have been achieved. When any one of these tests yielded a positive result, failure to cure *H pylori* infection was diagnosed.

Determination of **2* and **3* Polymorphisms of CYP2C19

The genotyping of the 2 mutated genes of CYP2C19, *CYP2C19*2* (**2*) (from guanine to adenine substitution at position 681 of exon 5) and *CYP2C19*3* (**3*) (from guanine to adenine substitution at position 636 of exon 4), was performed with the serial invasive signal amplification reaction assay according to previous reports[37,39,45] with minor modification. DNA was extracted from positive RUT samples by using the kit on the market (IsoQuick; Micro Probe Co, Garden Grove, CA). The primary probes and the invasive oligonucleotide for each SNP, which were designed to have theoretical annealing temperature of 63°C and 77°C, respectively, are listed in Table 1. The reactions were performed by using 384-well plates with reagents containing Cleavase XI enzyme, both F (FAM) dye and R (Redmond RED) dye (Epoch Biosciences, Redmond, WA), fluorescent resonance energy transfer (FRET) probes. In brief, 3 μL of each control or genomic DNA sample (≥40 ng DNA) was added into a 384-well microplate, and each well was overlayed with 6 μL mineral oil to prevent evaporation (Sigma Chemical Company, St Louis, MO). The plate was incubated at 95°C for 10 minutes,