**Helicobacter pylori eradication and its relation to antibiotic resistance and CYP2C19 status**


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**ABSTRACT**

Objective: to assess the efficacy of rabeprazole (RPZ), amoxicillin (Am), and clarithromycin (Cla) (7 vs. 14 days) in the eradication of *H. pylori*, and to determine the effect of strain-specific antibiotic resistance and host CYP2C19 status.

Material and methods: first, we determined the CYP2C19 status of 100 healthy subjects to establish a sample size for the clinical trial. Then, 59 *H. pylori*-infected patients were randomized to receive RPZ (20 mg daily) plus Cla (500 mg b.d.) and Am (1,000 mg cada 12 horas) for 7 vs. 14 days. The MIC for Am and Cla were determined using the agar dilution method. The CYP2C19 genotype was determined by the PCR-RFLP method.

Results: in the per-protocol analysis (PP) eradication rates were 89.7 and 72% for the 7- and 14-day groups (p = 0.159). In the intention to-treat analysis (ITT) eradication rates were 86.7 and 62.1% in the 7- and 14-day groups, respectively (p = 0.06). None of the strains was resistant to Am, and 4 strains were resistant to Cla: 3 (11.1%) in the 14-day group and 1 (4%) in the 7-day group. Neither strain-specific antibiotic resistance nor host CYP2C19 status influenced eradication rates.

Conclusions: both 7- and 14-day therapies were effective for *H. pylori* eradication. Strain resistance and CYP2C19 status do not seem to influence eradication rates in the studied population.

studies treatment fails in nearly 15% of patients with this therapy (1-5).

Several factors besides the well-established influence of antibiotic resistance have been related to this eradication failure, such as patient compliance, regimen selected, clinical presentation, and polymorphic cytochrome P450 2C19 (CYP2C19) status, which has been related to the metabolism of PPIs (6-15).

Allelic variants of CYP2C19 have been described: CYP2C19*2 and *3 alleles are associated with low CYP2C19 enzyme activity when compared to the CYP2C19 wild-type allele (CYP2C19*1). Thus, the various different genotypes of CYP2C19 affect the antisecretion action of PPIs, and consequently H. pylori eradication might be influenced (6,11,12,14).

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Individuals carrying 2 nonfunctional CYP2C19 alleles (*2/*2 or *3/*3) are called poor metabolizers (PMs), subjects with wild-type alleles in both exon 4 and exon 5 (*1/*1) are considered homozygous extensive metabolizers (Hom-EMs), and subjects with a single mutation in either exon 4 or exon 5 (*1/*2 or *1/*3) are considered heterozygous extensive metabolizers (Het-EMs).

The frequency of these phenotypes varies among different racial groups. Lower frequencies of PMs are found in Caucasians (1.8-2.8%) and black subjects (3.8%) versus Asian subjects (14.0%-14.3%), especially Japanese populations (21%) (16).

Several studies have demonstrated that the efficacy rates of H. pylori eradication are lower in Hom-EMs than in PMs or Het-EMs (8,9,13).

Our aim was to explore the influence of CYP2C19 genotypes and bacterial resistance in the success of H. pylori eradication.

METHODS

Determining the CYP2C19 genotype in the general population

Initially the CYP2C19 genotype was determined in the general population to establish a sample size for the clinical study. We included 100 subjects: F/M = 57/43, mean age = 44.9, born in North-Eastern Mexico, and with their ancestry –at least 2 generations– in the same geographical region. None of the subjects was related, and all were healthy and physically normal. A 5-ml sample of venous blood was collected from each subject, and genomic DNA was extracted by the phenol-chloroform-isoamyl alcohol and ethanol precipitation method.

Genotyping procedures for identifying the CYP2C19 wild-type (*1) gene and the two mutated alleles, *2 in exon 5 and *3 in exon 4, were performed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method with allele-specific primers, as described by de Morais et al. (20,21). All genotyping was performed at least twice. With these results patients were then classified in Hom-EMs, Het-EMs, and PMs as described previously.

Clinical study

Selection of the study population

We screened 112 patients at Hospital Universitario “Dr. José Eleuterio González”, Universidad Autónoma de Nuevo León, México, between May 2003 and April 2004. Patients with an indication of upper gastrointestinal endoscopy for dyspeptic symptoms such as epigastric pain, meal-related complaints, heartburn, nausea or vomiting were invited to participate.

We excluded patients with documented allergic reactions to medications used in this clinical trial; pregnant women; severely ill patients or patients with gastroesophageal reflux disease, peptic ulcer disease, or Zollinger-Ellison syndrome, and patients on a chronic regimen of non-steroid anti-inflammatory drugs. We also excluded patients with known hepatic, renal or cardiovascular disease, and patients treated with any antibiotic, PPI, or bismuth salts during the previous 4 weeks.

Patients that accepted to participate signed a written informed consent for participation. The local ethics committee approved this study protocol.

H. pylori status of patients assessed

H. pylori status was determined in all evaluated patients (n = 112) by culture, rapid urease test (RUT), and histology. H. pylori-positive status was based on two or more positive results.

During endoscopy, one biopsy from the antrum, one from the body, and one from the incisura angularis of the stomach were obtained for histology. One biopsy from the antrum and one from the corpus were obtained to perform a RUT using a non-commercial validated test (17). Two biopsies from the antrum and two from the corpus were obtained for culture, which was performed using standard procedures (18).

Patients included in the clinical study and treatment

Of all 112 patients screened, 59 patients were infected with H. pylori (F/M = 39/20, mean age = 38.5 years) and
were included in the clinical eradication study. In this protocol there was no follow-up for the 53 non-infected subjects.

Patients were then randomized in a 1:1 ratio to receive RPZ (20 mg daily) plus Cla (500 mg b.d.) and Am (1000 mg b.d.) for either 7 or 14 days. Treatment compliance was assessed by patient self-reports and tablet counts.

The minimal compliance to consider a patient valid for the analysis was at least 80%.

**CYP2C19 genotypes and determination of resistance to Am and Cla**

The CYP2C19 genotype was determined by the PCR-RFLP method as described for the general population in the first part of this study (20,21).

When the culture was positive for *H. pylori*, minimum inhibitory concentrations (MICs) were determined for Am and Cla (19). The cutoff values used to define resistance were ≥ 0.5 mg/l for Am and ≥ 2 mg/l for Cla.

**Detecting *H. pylori* eradication**

*H. pylori* eradication was defined by a negative result for stool antigen with monoclonal antibodies (Premier Platinum HpSA Plus, Meridian Diagnostics, Inc Cincinnati Ohio), which was performed 4 weeks after therapy completion.

**Statistics for the determination of the CYP2C19 genotype in the general population and the clinical study**

In the general population the genotypic distribution was examined for significant departures from the Hardy-Weinberg equilibrium by a chi-square test.

In the clinical study, for the efficacy analysis we used two approaches: a) an intention-to-treat (ITT) analysis, which was carried out on all patients who received at least one dose of the study drug, including patients in whom the eradication test was not performed by lack of a stool sample; and b) a per-protocol (PP) analysis, which included only patients that completed the regimen including the eradication test.

Statistical differences in eradication rates between study groups were evaluated using the analysis of variance. A two-sided p value < 0.05 was considered statistically significant.

The statistical programs used were Prism 3.0 (GraphPad Software, Inc, San Diego, Calif), and SPSS 11.0 (SPSS Inc, Chicago, IL).

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**RESULTS**

**CYP2C19 genotypes in the general population**

The studied population was in Hardy-Weinberg equilibrium for both polymorphic loci (exon 4, chi-square H-W = 1.8; exon 5, chi-square H-W = 1.42).

In all, 62% of subjects were Hom-EMs, 30% were Het-EMs, and 8% were PMs (Table I).

With these results we decided to include at least 29 patients per group, which allowed us to detect differences of 31% or greater in eradication rates (60% for 7-day group and 91% 14-day group, statistical power = 80%, and 5% α level).

**Table I. Distribution of CYP2C19 genotypes**

<table>
<thead>
<tr>
<th>CYP2C19 Genotype</th>
<th>General population (n = 100)</th>
<th>Clinical study (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exon 4</td>
<td>Exon 5</td>
</tr>
<tr>
<td>Hom-EMs</td>
<td>*1/*1</td>
<td>*1/*1</td>
</tr>
<tr>
<td>Het-EMs</td>
<td>*1/*1</td>
<td>*1/*2</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>*1/*3</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>*3/*3</td>
</tr>
<tr>
<td>PMs</td>
<td>*1/*2</td>
<td>*1/*3</td>
</tr>
<tr>
<td></td>
<td>*1/*1</td>
<td>*2/*2</td>
</tr>
<tr>
<td></td>
<td>*1/*3</td>
<td>*1/*2</td>
</tr>
<tr>
<td></td>
<td>*1/*2</td>
<td>*3/*3</td>
</tr>
</tbody>
</table>

Hom-EMs: homozygous extensive metabolizers; Het-EMs: heterozygous extensive metabolizers; PMs: poor metabolizers.

**Clinical trial**

**Compliance**

Of the 59 patients included in the clinical study 30 were included in the 7-day group and 29 in the 14-day group. In the 7-day group all patients showed a 100% compliance, while in the 14-day group compliance was 97%.

**H. pylori eradication**

The sample for the antigen stool test was collected in 29 of 30 patients in the 7-day group; regarding the 14-day group, 25 of 29 patients collected the stool sample.

In the PP analysis, which included all patients who completed the study and collected stool samples for *H. pylori* eradication identification, the eradication rate was 89.7% (95% CI = 72.7-97.8) for the 7-day treatment and 72% (95% CI = 50.6-87.9) for the 14-day group (p = 0.159) (Table II).
**Eradication rates for both study groups and distribution of clarithromycin resistance**

<table>
<thead>
<tr>
<th>Group</th>
<th>Per protocol (p = 0.159)</th>
<th>Intention-to-treat (p = 0.06)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td>89.7 (72.7-97.8)</td>
<td>86.7 (69.3-96.2)</td>
</tr>
<tr>
<td></td>
<td>26/29</td>
<td>26/30</td>
</tr>
<tr>
<td>14 days</td>
<td>72 (50.6-87.9)</td>
<td>62.1 (42.3-79.3)</td>
</tr>
<tr>
<td></td>
<td>18/25</td>
<td>18/29</td>
</tr>
</tbody>
</table>

In the ITT analysis, which included all patients who received at least one dose of study drugs, including patients who did not collect any stool samples for the detection of *H. pylori* eradication, the eradication rate was 86.7% in the 7-day group (95% CI = 69.3-96.2), whereas in the 14-day group was 62.1% (95% CI = 42.3-79.3) (p = 0.06).

### Effect of strain resistance on *H. pylori* eradication

We obtained a positive culture in 52 (88.1%) of all 59 patients included (25 from the 7-day group and 27 form the 14-day group). None of the strains had resistance to Am, while 4 strains were resistant to Cla: 3 (11.1%) in the 14-day group and 1 (4%) in the 7-day group.

When we performed the analysis considering only patients infected with susceptible strains and who had completed the study we found that eradication rates were 91.7% (22/24) (95% CI = 0.73-0.99) for the 7-day group and 79% (15/19) (95% CI = 0.56-0.92) for the 14-day group (p = 0.38).

### Effect of *CYP2C19* genotypes in *H. pylori* eradication

In the clinical study, 43 (72.9%) patients were *1/*1 for both polymorphic sites (Table I). In the PP analysis there were no differences in the 7- vs. 14-day therapies regarding *CYP2C19* status (Table III).

### DISCUSSION

A better understanding of molecular grounds to explain ethnic differences in metabolism and response to different drugs could contribute to improve individualized pharmacotherapy. In this study we determined the prevalence of *CYP2C19* genotypes, studied the influence of these genotypes in eradication rates using a triple therapy (RPZ plus Am and Cla, 7 vs. 14 days), and correlated these data with strain resistance.

In the general population we found that 62% of subjects were Hom-EMs, 30% were Het-EMs, and 8% were PMs. The value for PMs was smaller than reported for the Chinese population (13.8%), but higher to that reported in blacks (3.8%) and Caucasians (2.1%) (16), which suggests an Asian component present in the Mexican population.

With respect to the clinical trial, the 7-day therapy was as effective as the 14-day therapy for the eradication of *H. pylori* in both the PP and ITT analyses. In fact, contrary to the expected higher eradication rate with the 14-day therapy, this study group had a lower eradication rate (72%) versus the 7-day therapy (89.7%), and these differences almost reached significance in the ITT analysis (p = 0.06). It should be noted that in the 14-day group there was a higher frequency of *H. pylori* resistance versus the 7-day group (11 vs. 3.7%), and this fact could explain this non-significant difference.

Polymorphism in *CYP2C19* is the main cause for the large interindividual variability in the pharmacokinetics of PPIs. Several trials demonstrated that cure rates for *H. pylori* infection are affected by *CYP2C19* status (8,9,13). We did not confirm these findings. It seems that in the population studied this fact is not a decisive factor for eradication success, at least with the combinations of drugs and doses used in this trial.

It has been described that in so-called PM and Het-EM individuals drug exposure is about 5 and 3-times higher, respectively, than in EMs. The inhibition of acid secretion is clearly related to drug exposure, and clinical efficacy depends on the extent and duration that intragastric pH is increased, thus the therapeutic outcome is associat-
ed with each individual genotype (phenotype) of CYP2C19 (6,11,12,14). However, this is not the only fact affecting eradication: other factors such as compliance could modify response to therapy. In this study compliance was documented by pill counts only, which is simple but not exempt of non-intentional errors.

This study found no evidence that CYP2C19 genotypes or H. pylori antibiotic resistance may affect eradication rates in this particular population. A possible explanation for the lack of correlation found in this study is that the prevalence of resistant strains was quite low (7.7%), in fact slightly lower than the previously observed rate for the same population (12.9%) (22), and this fact could contribute to the low impact of bacterial resistance on eradication rates.

This study showed that the efficacy of our treatment (RPZ plus Am and Cla) is as good for 7 as for 14 days—even using low doses of RPZ (20 mg daily).

In this protocol two efficacy analyses were performed: an ITT analysis, which was performed considering all patients who received at least one dose of drugs, including patients who did not collected any stool samples for the detection of H. pylori eradication; and a PP analysis, which only included patients who completed the regimen and collected their stool samples for the detection of H. pylori eradication. According to the information obtained, the PP analysis is more accurate, as in the ITT analysis it is possible that patients who completed the regimen and collected no stool samples had their bacteria actually eradicated, and this fact could have changed the percentages of eradication obtained.

In conclusion, both 7- and 14-day therapies using RPZ-Cla-Am were effective for H. pylori eradication. We did not find any differences in strain resistance or CYP2C19 status between both regimens used.

REFERENCES