A comparison of the acid-inhibitory effects of esomeprazole and rabeprazole in relation to CYP2C19 polymorphism

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ABSTRACT

Background
Esomeprazole and rabeprazole are metabolized in the liver with involvement of the polymorphic CYP2C19 enzyme. This functional genetic polymorphism determines enzyme activity. Among Caucasians, 70% of the population has a fast metabolizer phenotype, 25-30% an intermediate, and 2-5% a slow metabolizer phenotype.

Aim
To compare the acid-inhibitory effects of esomeprazole 40 mg and rabeprazole 20 mg at 4, 24, and 120 hours after oral administration in relation to CYP2C19 genotype.

Methods
CYP2C19*2 to *6 and *17 genotypes were determined in healthy H. pylori-negative Caucasian subjects. Eighteen subjects (mean age 21y, 7 male) with different genotypes (7 wt/wt, 7 wt/*2, 2 wt/*17 and 2 *2/*17) were included in a randomized investigator-blinded cross-over study with esomeprazole 40 mg and rabeprazole 20 mg. Intragastric 24-h pH-monitoring was performed on days 0, 1 and 5 of oral dosing.

Results
Onset of acid-inhibition during the first 4 hours after administration did not differ significantly between esomeprazole and rabeprazole. During the upright period, percentage of time with pH > 4 was significantly increased with esomeprazole compared to rabeprazole (52.2 vs. 40.3, $P = 0.003$).

At day 1 and 5, acid-inhibition with esomeprazole was significantly greater than with rabeprazole (median intragastric pH: day 1: 3.7 vs. 3.0, $P = 0.008$; day 5: 4.7 vs. 3.8, $P = 0.000$; percentage of time pH > 4: day 1: 45% vs. 39%, $P = 0.054$; day 5: 65% vs. 48% $P = 0.000$). Differences in acid-inhibition between wt/wt and wt/*2 genotype were significant for both PPIs.

Conclusions
Once-daily dosing with esomeprazole 40 mg orally provides a more effective and faster acid-inhibitory effect than rabeprazole 20 mg orally. Esomeprazole shows a higher rate of responders after single and multiple dosing than rabeprazole. Acid-inhibition of both esomeprazole and rabeprazole is influenced by CYP2C19 polymorphism.
INTRODUCTION

Rabeprazole and esomeprazole are claimed to be the fastest and most potent available proton pump inhibitors (PPIs) [1-6]. Compared with the other PPIs, rabeprazole is less dependent on low pH for conversion to its active form owing to its higher pKa, approximating 5, while other PPIs have a pKa ~4 or lower. This means that rabeprazole undergoes rapid activation over a wider pH range. These characteristics suggest that it should produce a more rapid onset of acid-inhibition than the other PPIs [7-9].

Both esomeprazole and rabeprazole are metabolized in the liver by the cytochrome P450 (CYP) enzyme CYP2C19, rabeprazole is also non-enzymatically metabolized [10-13].

The CYP2C19 enzyme has several functional polymorphisms. Subjects with non-mutated variants for CYP2C19 are referred to as wildtype/wildtype (wt/wt or *1/*1) genotype which corresponds with a homozygous extensive metabolizer phenotype. When subjects possess one of the CYP2C19*2 to *6 variant alleles, their genotype is known as wt/*2 (or wt/*3, or wt/*4 etc), corresponding with a heterozygous extensive metabolizer phenotype.

With two mutated variants, the genotype can be *2/*2 (or *2/*3 or *3/*3 etc), corresponding with a poor metabolizer phenotype. The *2 to *6 variants are associated with reduced metabolism of omeprazole, leading to higher systemic availability reflected by higher blood levels (and/or higher area under the concentration curves (AUCs)) and thus more profound acid inhibition [14-16]. In contrast to *2 to *6 variants, *17 variants are associated with increased metabolism of omeprazole. The *17 allele refers to ultrarapid metabolizers (wt/*17 or *17/*17 genotype), resulting in lower blood levels (and/or lower AUCs) and reduced acid inhibition [17, 18]. The prevalence of CYP2C19 mutations differs among populations. Asian subjects have a higher prevalence of *2 and *3 alleles than Caucasians. In the Caucasian population, about 40% has a wt/wt genotype, about 25% has a wt/*2 genotype and 3% has a *2/*2 genotype [19]. In the Chinese population, about 50% has a wt/wt genotype, about 40% has a wt/*2 or wt/*3 and 12% has a *2/*2, *2/*3 or *3/*3 genotype. The CYP2C19*17 allele has an opposite geographic distribution. About 25% of the Caucasian population has a wt/*17 or *17/*17 genotype compared to 1% of the Chinese and the Japanese population [17, 20]. While the effects of CYP2C19 genotypes on the metabolism and acid suppressive effects of omeprazole are consistently reported, reports on the effect on rabeprazole are inconsistent. Some studies reported an influence of CYP2C19 polymorphism [21-23], whereas other studies did not [24, 25]. These studies were carried out in Asian subjects. In previous studies with esomeprazole, no influence of CYP2C19 genotype on the acid-suppressive effect and pharmacokinetics was observed [1, 26]. The first study explored the effect of CYP2C19 in Caucasian homozygous (wt/wt) and heterozygous extensive (wt/*2) metabolizers [1] and the latter study investigated the influence of CYP2C19 in Chinese extensive and poor metabolizers [26].

Most comparisons of the effects of PPI treatment on intragastric pH were performed at day 1 (24 hours after administration, effect of single dose), or at day 5 (120 hours after administration, effect during steady state). There are, however, very few published studies of the acid suppressive effects of PPIs at other points in time, in particular during the first hours after oral administration. This is clinically relevant as many patients nowadays use PPIs on a non-continuous basis [27]. Short intermittent treatment or on-demand therapy with a PPI requires an agent that has a rapid and sustained onset of action after a single dose.

The objective of this study therefore was to compare the acid-inhibitory effects of esomeprazole 40 mg and rabeprazole 20 mg at 4, 24 (including day and night period) and 120 hours after oral administration in a Caucasian population of H. pylori-negative subjects with known CYP2C19 genotype.
MATERIALS AND METHODS

Study design
A randomized, single centre, two-way cross over, investigator-blinded study was performed in the Haga Teaching Hospital between August 2004 and January 2007. After inclusion each subject was assigned to one of the two 5-day dosing periods during which the subject received either oral esomeprazole 40 mg once daily (o.d.) or oral rabeprazole 20 mg o.d. Dosing periods were separated by washout periods of at least 14 days. The effect of both drugs on intragastric acidity was assessed by 24-h intragastric pH monitoring on day 1 and day 5 of administration. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The institutional review board of the Haga Teaching Hospital approved the study protocol and all subjects gave written informed consent. The subjects were allocated to a treatment regimen according to a randomised cross over sequence, given by a computer generated randomisation list.

Sample Size
The power calculation was based on parametric assumptions. The primary outcome variable was percentage of time (during 24 hours) that pH is larger than 4. This variable was compared between two treatments (40 mg esomeprazole and 20 mg rabeprazole) in a 2-periods 2-treatments cross-over study. A clinically relevant mean difference of the outcome variable between the two treatments was 10 percent points. The standard deviation of the outcome variable was set at 16 percent points [28]. Assuming a Pearson correlation of 0.54 between the two measurements under consecutive treatments [29], the above clinically relevant mean difference was detectable with 80% power in 18 subjects, given a test size alpha of 0.05 (2-sided). To study the effect of CYP2C19 genotype on the inhibition of gastric acid secretion by esomeprazole and rabeprazole, the study population was composed of nine homozygous extensive metabolizers and nine heterozygous extensive metabolizers.

Subjects
Subjects were aged between 18 and 35 years, with normal physical examination and laboratory screening tests (haemoglobin, white blood cell total count, serum blood glucose, serum creatinine, total bilirubin, serum alkaline phosphatase, serum ASAT and ALAT). They were eligible for inclusion if an H. pylori urea breath test was negative, if their 24-h baseline intragastric pH measurement had a pH < 4 for more than 70% of the time (more than 16.8 h), and if their CYP2C19 genotype was known. Individuals were excluded from the study if they were pregnant, if they had gastrointestinal disorders that might impair drug absorption, if they had a body mass index (BMI) with a deviation of more than 15% of normal (normal values: BMI 18.5-25 [30]) or if they had a history of alcohol or drug abuse. Except for oral contraceptives and the occasional use of paracetamol (acetaminophen), subjects took no other drugs than the study medication.

Test days protocol
During the days of pH monitoring, subjects stayed in the clinic in a special research room. Subjects with negative H. pylori urea breath test and known CYP2C19 status arrived at the pH laboratory of the clinic by 08:30 hours. pH measurements were performed as previously described [28, 31]. pH recordings started at 08:55 hours (day 0). The following day (day 1) pH recording continued for 24 hours if intragastric pH was below pH 4 for more than 70% of the time during day 0 (baseline). The subjects got the first dose of the study medication five minutes before standard breakfast. From 23:00h the subjects remained
in fasting condition and slept. They arose again between 07:00 and 07:30h the next day. The pH electrode was removed at 08:55h (day 2) and the position of the assembly was checked prior to removal.

At day 5, the subjects returned at the pH-laboratory and their personal pH-electrode was again inserted and positioned for 24-h intragastric pH monitoring (steady state). From 23:00 hours the subjects remained in fasting condition and slept. They arose again between 07:00 and 07:30h the next day. The pH electrode was removed at 08:55h (day 6) and the position of the assembly was checked prior to removal. Standard meals and drinks were provided as previously described [28, 31].

**Intragastric pH monitoring**

Intragastric pH was measured by miniature glass electrode with internal reference (diameter 3 mm, model 440M3, Mettler Toledo, Urdorf, Switzerland) connected to a portable datalogger with an exchangeable 96 Kb memory (GastrograpH Mark II, SME Medizintechnik GmbH, Weil am Rhein, Germany). The sampling rate of these dataloggers is 4 per second. Every two seconds, the median of 8 voltage measurements is calculated and stored in the memory (RAM). After completion of post-measurement calibration the raw measurement data were transferred to a personal computer.

Data analysis and statistics were based on median pH values over 6 seconds.

**CYP2C19 genotyping**

Genotyping procedures identifying CYP2C19 wild-type gene and the variant alleles, *2 to *6 and *17 were performed using the CYP2C19 LightCycler kit (Roche, Mannheim, Germany) at the Department of Clinical Chemistry, Erasmus MC University Medical Center, Rotterdam, the Netherlands.

**Pharmacodynamic data**

To assess the effect of both proton pump inhibitors on day 1 and 5 of administration two pH parameters were calculated: median pH values over predefined time periods and cumulative percentages of time that intragastric pH values were above pH 4 over these time periods. Predefined time periods: first 4 hours after dosing, first 24 hours (day 1) and last 24 hours (day 5) with day and night periods. Night was defined as the time period in the supine position. Day was defined as the time during the upright position.

To determine the net response to the study drug, the cumulative percentage of time with pH above threshold 4 at baseline was subtracted from the cumulative percentage of time with pH above threshold 4 at day 1 and day 5 for each individual subject [32]. This gain is represented as Δ % of time with intragastric pH > 4. A change in this Δ % of time of less than 10% was considered as a non-response, given the accuracy of the technique of intragastric pH monitoring and the variability in 24-h intragastric acidity [33]. We defined individuals showing a Δ of ≥ 10% as responders and individuals with a Δ of < 10% as non-responders.

**Statistical analysis**

Statistical comparison between esomeprazole and rabeprazole administration was done by a mixed model ANOVA with restricted maximum likelihood estimates for the effects. Complete cases (n=18, intention to treat) with all six repeated measurements (possibly containing missing values) were analysed parametrically. A compound symmetry structure was imposed on the 6 x 6 (co)variance matrix. Missing values were appropriately dealt with by using the maximum likelihood estimation procedure. In the model 8 parameters were estimated: 6 for the time effect (2 periods times, 3 days per period) and 2 for the treatment effect (esomeprazole – rabeprazole at days 1 and 5). Median pH values over the whole 24-h period, day- and night-time, and cumulative percentages of time during which pH
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was above pH 4 over these time periods were compared. The mixed model ANOVA was also used to determine the effect of CYP2C19*2 mutation on acid inhibition with esomeprazole and rabeprazole. Statistical comparison between wt/wt and wt/*2 under either treatment was made in complete and incomplete cases. Median pH values over the whole 24-h period, day- and night-time, and cumulative percentages of time during which pH was above pH 4 over these time periods were compared.

RESULTS

Eighteen healthy subjects (7 male and 11 female, with a mean age of 21 (range 18 – 27) years, and a mean body mass index of 21.8 (19.6 -24.4) kg/m²) were included in the study. All subjects completed the study. Seven had a wt/wt genotype, 7 were wt/*2, 2 were wt/*17 and 2 had a *2/*17 genotype. No *3 to *6 mutations were observed. Both drugs were well tolerated and there were no clinically relevant adverse events reported. Percentages of time with pH > 4 during baseline are shown in Table 1A.

Table 1A Mean (95% CI) of the % of time that the intragastric pH was > 4 at day 0 of both study periods in the total group of 18 subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 0</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period 1</td>
<td>Period 2</td>
<td></td>
</tr>
<tr>
<td>% pH &gt; 4 24h</td>
<td>12.4 (8.3 to 16.5)</td>
<td>9.5 (5.6 to 13.3)</td>
<td></td>
</tr>
<tr>
<td>% pH &gt; 4 U*</td>
<td>8.4 (6.0 to 10.8)</td>
<td>8.2 (4.3 to 12.0)</td>
<td></td>
</tr>
<tr>
<td>% pH &gt; 4 S*</td>
<td>20.1 (10.4 to 29.8)</td>
<td>12.0 (3.3 to 20.7)</td>
<td></td>
</tr>
</tbody>
</table>

*U: upright, S: supine

For the parametric analysis of the data over the first 4-h period after the first dosing median pH data needed a ln transformation and the percentages of time below or above pH threshold 4 a logit transformation. Median intragastric pH over the first 4-h period with esomeprazole was 2.27 and with rabeprazole 1.85 (Figure 1). Although intragastric pH with rabeprazole was 18.5% lower (95% CI: -39.3 to 9.5) the difference was not significant (P = 0.16). With esomeprazole the percentage of time with intragastric pH > 4 was 16.6% and with rabeprazole 6%. This difference was not significant (P = 0.13 with an odds ratio of 3.12 (0.69 to 14.12)).

Figure 1 Median intragastric pH (over 10-minute time intervals) in the upright hours after dosing of esomeprazole 40 mg and rabeprazole 20 mg at day 1 (n =18)
At day 1 of administration during the upright period, median intragastric pH of esomeprazole treatment for the total group of subjects did not differ significantly from rabeprazole. The percentage of time with an intragastric pH > 4 was significantly higher with esomeprazole than with rabeprazole (Table 1B and 1C). During the 24-h period, median intragastric pH of esomeprazole treatment was significantly higher than with rabeprazole (Table 1B and 1C and Figure 2). With esomeprazole, 16 out of 18 subjects (89%) and with rabeprazole, 14 out of 18 subjects (78%) showed a response of ≥ 10% at day 1. At day 5, median intragastric pH and the percentage of time with an intragastric pH > 4 of esomeprazole were significantly higher than rabeprazole during both the upright period and the 24-h period (Table 1B and 1C). At day 5, all subjects in the esomeprazole group (100%) and 17 out of 18 subjects (94%) in the rabeprazole group showed a response of ≥ 10%.

Figure 2 Individual (n=18) and mean values of percentage of time with intragastric pH > 4 during the 24-h period at day 0, day 1 and day 5 of administration of esomeprazole 40 mg (E) and rabeprazole 20 mg (R)

Table 1B Mixed model ANOVA estimates (95% CI) of the mean levels of median pH and % pH > 4 during treatment, adjusted for a possibly confounding time effect, in the total group (n=18)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 1</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>esomeprazole</td>
<td>rabeprazole</td>
</tr>
<tr>
<td>median pH 24h</td>
<td>3.7 (3.1 - 4.2)</td>
<td>3.0 (2.4 - 3.5)</td>
</tr>
<tr>
<td>median pH U*</td>
<td>3.8 (3.2 - 4.4)</td>
<td>3.3 (2.7 - 3.9)</td>
</tr>
<tr>
<td>median pH S*</td>
<td>3.0 (2.4 - 3.7)</td>
<td>3.2 (2.5 - 3.8)</td>
</tr>
<tr>
<td>% pH &gt; 4</td>
<td>45.4 (36.8 - 54.0)</td>
<td>39.0 (30.4 - 47.6)</td>
</tr>
<tr>
<td>% pH &gt; 4 U</td>
<td>52.2 (42.5 - 62.0)</td>
<td>40.3 (30.7 - 50.0)</td>
</tr>
<tr>
<td>% pH &gt; 4 S</td>
<td>33.1 (21.0 - 45.2)</td>
<td>36.4 (24.3 - 48.5)</td>
</tr>
</tbody>
</table>

*U: upright, S: supine
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**Table 1C** Mixed model ANOVA estimates (95% CI) of the treatment effects (esomeprazole-rabeprazole) in the total group (n=18)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 1</th>
<th></th>
<th>Day 5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>effect</td>
<td>95 % CI</td>
<td>P</td>
<td>effect</td>
</tr>
<tr>
<td>median pH 24h</td>
<td>0.68</td>
<td>0.2 - 1.1</td>
<td>0.008</td>
<td>0.86</td>
</tr>
<tr>
<td>median pH U*</td>
<td>0.50</td>
<td>-0.03 - 1.1</td>
<td>0.062</td>
<td>1.23</td>
</tr>
<tr>
<td>median pH S*</td>
<td>-0.16</td>
<td>-0.6 - 0.3</td>
<td>0.440</td>
<td>0.15</td>
</tr>
<tr>
<td>% pH &gt; 4</td>
<td>6.4</td>
<td>-0.1 - 12.9</td>
<td>0.054</td>
<td>16.3</td>
</tr>
<tr>
<td>% pH &gt; 4 U</td>
<td>11.9</td>
<td>4.7 - 19.1</td>
<td>0.003</td>
<td>23.4</td>
</tr>
<tr>
<td>% pH &gt; 4 S</td>
<td>-3.3</td>
<td>-14.0 - 7.4</td>
<td>0.518</td>
<td>-0.33</td>
</tr>
</tbody>
</table>

*U: upright, S: supine

During esomeprazole administration, heterozygous carriage of a CYP2C19*2 mutation resulted in significantly higher median intragastric pH at day 1 and a significantly higher percentage of time with intragastric pH > 4 at day 1 and 5 (Figure 3 and Table 2A). During administration of rabeprazole, significant differences between wt/wt and wt/*2 genotypes were observed in the percentage of time with intragastric pH > 4 at day 1, but not in median intragastric pH. At day 5, a significant difference was found in median intragastric pH (Figure 3 and Table 2B) between genotypes. For both esomeprazole and rabeprazole, significant differences in 24-h median intragastric pH between wt/wt and wt/*2 were observed during the upright period and not during the supine period (Table 2A and B).

**Figure 3** Individual and mean values of percentage of time with intragastric pH > 4 (left) and median intragastric pH (right) in wt/wt (n=7) and wt/*2 (n=7) subjects after administration of esomeprazole 40 mg or rabeprazole 20 mg at day 1 and day 5

**Day 1:**
**Day 5:**

![Graph showing comparison of intragastric pH over time for esomeprazole and rabeprazole](image)

**Table 2A** Mixed model ANOVA estimates (95% CI) of the mean levels of median pH and treatment effect (wt/wt – wt/*2) on day 1 and 5 of administration of esomeprazole (n=18)

<table>
<thead>
<tr>
<th></th>
<th>day 1</th>
<th>wt/wt</th>
<th>wt/*2</th>
<th>effect</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>median pH 24h</td>
<td>3.1 (2.4 - 3.9)</td>
<td>4.7 (3.9 - 5.5)</td>
<td>-1.57</td>
<td>-2.65 - -0.5</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>median pH U*</td>
<td>3.3 (2.4 - 4.15)</td>
<td>4.5 (3.6 - 5.4)</td>
<td>-1.24</td>
<td>-2.5 - 0.01</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>median pH S*</td>
<td>2.75 (1.8 - 3.7)</td>
<td>3.75 (2.8 - 4.7)</td>
<td>-1.0</td>
<td>-2.4 - 0.4</td>
<td>0.142</td>
<td></td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median pH 24h</td>
<td>4.2 (3.6 - 4.85)</td>
<td>5.0 (4.35 - 5.6)</td>
<td>-0.75</td>
<td>-1.6 - 0.13</td>
<td>0.089</td>
<td></td>
</tr>
<tr>
<td>median pH U*</td>
<td>4.9 (4.4 - 5.45)</td>
<td>5.4 (4.9 - 5.95)</td>
<td>-0.5</td>
<td>-1.3 - 0.3</td>
<td>0.184</td>
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<tr>
<td>median pH S*</td>
<td>3.0 (1.7 - 4.3)</td>
<td>4.3 (3.0 - 5.6)</td>
<td>-1.3</td>
<td>-3.2 - 0.6</td>
<td>0.165</td>
<td></td>
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</table>

*U: upright, S: supine

**Table 2B** Mixed model ANOVA estimates (95% CI) of the mean levels of median pH and treatment effect (wt/wt – wt/*2) on day 1 and 5 of administration of rabeprazole (n=18)

<table>
<thead>
<tr>
<th></th>
<th>day 1</th>
<th>wt/wt</th>
<th>wt/*2</th>
<th>effect</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>median pH 24h</td>
<td>2.5 (1.7 - 3.25)</td>
<td>3.4 (2.6 - 4.15)</td>
<td>-0.89</td>
<td>-1.97 - -0.19</td>
<td>0.102</td>
<td></td>
</tr>
<tr>
<td>median pH U*</td>
<td>2.7 (1.8 - 3.6)</td>
<td>4.0 (3.15 - 4.9)</td>
<td>-1.32</td>
<td>-2.6 - 0.08</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>median pH S*</td>
<td>2.8 (1.9 - 3.8)</td>
<td>4.0 (3.0 - 5.0)</td>
<td>-1.18</td>
<td>-2.55 - 0.2</td>
<td>0.088</td>
<td></td>
</tr>
<tr>
<td></td>
<td>day 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median pH</td>
<td>3.4 (2.8 - 4.05)</td>
<td>4.4 (3.75 - 5.0)</td>
<td>-0.95</td>
<td>-1.8 - -0.06</td>
<td>0.037</td>
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<tr>
<td>median pH U*</td>
<td>3.5 (3.0 - 4.1)</td>
<td>4.45 (3.9 - 5.0)</td>
<td>-0.93</td>
<td>-1.7 - -0.16</td>
<td>0.020</td>
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<tr>
<td>median pH S*</td>
<td>3.2 (1.9 - 4.5)</td>
<td>3.75 (2.4 - 5.0)</td>
<td>-0.56</td>
<td>-2.4 - 1.3</td>
<td>0.541</td>
<td></td>
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</table>

*U: upright, S: supine
DISCUSSION

The aim of the study was a comparison of the acid-inhibitory effects of esomeprazole 40 mg and rabeprazole 20 mg in relation to CYP2C19 polymorphism. Therefore, the intragastric pH studies were carried out in a population of healthy \textit{H. pylori}-negative subjects.

It has been argued that the higher pKa of rabeprazole would account for its faster onset of action than lansoprazole, omeprazole, and pantoprazole [6, 7, 34]. Data from previous studies that compared single doses of rabeprazole and esomeprazole in healthy subjects showed a faster increase in intragastric pH during the upright period than rabeprazole [35, 36]. Our data showed no significant difference between onset of action between esomeprazole and rabeprazole during the first 4 hours after administration. There was a tendency to a better acid-inhibitory effect during the upright period (e.g. the first 14 hours after administration) with esomeprazole. During the supine period, others observed a significantly increased acid-inhibitory effect of rabeprazole [35, 36]. We observed no difference in acid-inhibition between esomeprazole and rabeprazole during this period.

Median intragastric pH over the first 24-h post-dosing period was significantly higher with esomeprazole than with rabeprazole. At 120-h post-dosing, the median 24-h intragastric pH and the percentage of time with intragastric pH > 4 were significantly higher with esomeprazole. Two previous studies in healthy volunteers reported equivalence between esomeprazole 40 mg and rabeprazole 20 mg in mean percentage of time with intragastric pH > 4 (esomeprazole vs. rabeprazole 45.4 vs 44.0\% [36], and 45.2 vs. 45.3\% [35]) after a single dose. Unfortunately, these studies only showed derivative parameters (AUC intragastric pH and percentage of time with pH > 4), rather than median 24-h intragastric pH data. Furthermore, these studies used antimony pH electrodes. These electrodes are known to be less precise than glass electrodes, especially during intragastric pH monitoring, making it more difficult to measure small differences between PPIs [37]. One other study showed data of esomeprazole 40 mg and rabeprazole 20 mg after 5 days of dosing that are comparable to our data (median intragastric pH of esomeprazole 4.3 vs. rabeprazole 3.5, mean percentage of time with an intragastric pH > 4 with esomeprazole 61\% vs. rabeprazole 45\%) [3]. Two studies were performed in patients with symptoms of GERD [38, 39]. These studies showed that esomeprazole 40 mg provided greater acid control in more patients and maintained intragastric pH for a longer period of time above 4 than rabeprazole 20 mg. In the above mentioned studies, the acid-inhibitory effects of both PPIs were not investigated in relation to pharmacokinetics and pharmacogenetics and only two studies measured baseline pH [35, 36]. By studying baseline intragastric pH, the percentage of responders can be calculated. We found that 11\% did not respond after a single dose of esomeprazole, vs. 22\% of the same subjects showing no response after rabeprazole.
Our study has demonstrated that the pharmacodynamics of esomeprazole and rabeprazole are influenced by CYP2C19 genotype in \textit{wt/wt} and \textit{wt/*2} subjects. For rabeprazole, this pharmacogenetic influence has been shown before, mainly in Asian subjects [21, 22, 24, 40, 41]. It is remarkable that the differences in acid-inhibition between \textit{wt/wt} and \textit{wt/*2} genotype were mainly observed during the upright and 24-hour period and not during the supine period. Two factors may account for this finding. At first, monitoring of intragastric pH during the supine period can be susceptible to larger variability in pH data due to duodenogastric reflux of alkaline origin [42, 43]. At second, we know from in vitro inhibition studies that the concentration of proton pump inhibitor surrounding the CYP2C19 receptor lies in the range of the $K_i$ [44]. The $K_i$ is a parameter that accounts for 50% inhibition of the CYP enzyme. At moments after drug intake when higher serum concentrations are achieved (e.g. the first couple of hours after administration and first pass mechanism resulting in higher concentrations in the liver) inhibition of CYP2C19 will be optimal because of the concentration of the drug will be higher than the $K_i$. During clearance of the drug, this effect will reverse: the $K_i$ will not be reached anymore and inhibition of CYP2C19 will disappear. In this perspective, it would be interesting to investigate the influence of CYP2C19 on \textit{wt/wt} and \textit{wt/*2} genotypes with a twice daily dosing schedule of esomeprazole and rabeprazole.

For esomeprazole, the results between \textit{wt/wt} and \textit{wt/*2} genotypes are not in line with data from previous studies [1, 26, 45]. Two of these studies had a different design or objective than our studies. One open, randomized crossover study was designed to evaluate the effect of single and repeated administration of esomeprazole 40 mg on intragastric pH in healthy Chinese extensive metabolizers (EMs) (no division was made between homEMs and hetEMs) compared with PMs. On genotype analysis, 28 of the subjects were EM and eight were PM. Those who were PM tended to have a higher, albeit not statistically significant, percentage of time with intragastric pH $> 4$ and the median 24-h intragastric pH than those who were EM [26]. In another study, it was tested whether esomeprazole-induced healing of GERD is related to CYP2C19 genotype. The results showed that the frequency distribution of CYP2C19 genotypes was not different between patients with complete and incomplete healing [45]. The conflicting results of the influence of CYP2C19 between this study and our previous study, may be caused by small differences in acid-suppressive response between subjects with \textit{wt/wt} and \textit{wt/*2} genotypes. Although the studies were identical in design and powered to detect significant differences between \textit{wt/wt} and \textit{wt/*2} genotype, a type II error could have occurred. A larger prospective study is warranted.

**CONCLUSION**

Once-daily dosing with esomeprazole 40 mg orally provides a more effective and faster acid-inhibitory effect than rabeprazole 20 mg orally. Esomeprazole shows a higher rate of responders after single and multiple dosing than rabeprazole. Acid-inhibition of both esomeprazole and rabeprazole is influenced by CYP2C19 polymorphism.
REFERENCES


Determination of rabeprazole and metabolite in human serum using high-speed HPLC

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Determination of rabeprazole and metabolite in human serum using high-speed HPLC

ABSTRACT

Aim
To develop a high-speed, high performance liquid chromatography (HPLC) method for the determination of concentrations of rabeprazole and its metabolite rabeprazole thio-ether in the serum of Caucasian individuals.

Methods
Serum concentrations of rabeprazole and rabeprazole thio-ether were determined by liquid-liquid extraction and HPLC with a rapid resolution column. Accuracy and precision of intra-day and inter-day variation, linearity, the lower limit of quantitation (LLOQ), recovery and sample stability were determined as validation parameters.

Results
The LLOQ was 0.015 mg/L rabeprazole (n = 6, coefficient of variation (CV), 11.9%) and 0.026 mg/L rabeprazole thio-ether (n = 6, CV 12.6%) in human serum. Calibration curves were established between 0.015-1.4 mg/L for rabeprazole and 0.026-0.5 mg/L for rabeprazole thio-ether by non-weighted linear regression. The inter-day correlation coefficients of rabeprazole and its thio-ether were 0.999 or greater. The precision showed a CV of < 0.43%, the bias of intra-day variation was < 11.6% and the bias of inter-day variation was < 12.6%, each tested with n = 6. The recovery from calf serum of rabeprazole was 75.7% and of rabeprazole thio-ether 99.9%. The accuracy in calf serum showed a CV of < 7.2%. In human serum samples the accuracy was 100.9% for rabeprazole and 98.1% for rabeprazole thio-ether, each tested with n = 6. Frozen quality control samples were stable for at least six months (deviation < 5%).

Conclusion
Quantitation of rabeprazole and rabeprazole thio-ether by high-speed HPLC method is very fast (a run time < 1.5 minutes), accurate and precise. The method is appropriate for a rapid determination of serum concentrations, especially when there is a large number of samples requiring analysis.
INTRODUCTION

Rabeprazole, 2-([(4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl)sulphinyl]-1H-benzimidazole (Figure 1), a substituted benzimidazole, like omeprazole, is a selective PPI. Rabeprazole is approved for the treatment of gastro-oesophageal reflux disease with or without oesophagitis, erosive oesophagitis, gastric hypersecretion and duodenal ulcer disease, and eradication of *H. pylori* infection in combination with amoxicillin 1,000 mg and clarithromycin 500 mg [1].

Figure 1 Chemical structure of lansoprazole (internal standard), rabeprazole and rabeprazole thio-ether

PPIs are pro-drugs that are activated by conversion to sulphonamides in the acidic environment of the caniculum of the parietal cells of the stomach. The metabolism of rabeprazole, like omeprazole, is regulated by an enzyme of the cytochrome P450 system in the liver, CYP2C19 [2]. Metabolites are rabeprazole thio-ether, rabeprazole sulphone and desmethyl rabeprazole. Rabeprazole and rabeprazole thio-ether are pharmacologically active substances.

Like omeprazole, higher rabeprazole AUCs are observed in CYP2C19 poor metabolizers compared with homozygous and heterozygous extensive metabolizers [2, 3]. To investigate the effect of CYP2C19 genotype status on the pharmacokinetics of rabeprazole in Caucasian subjects, a large number of serum samples from pharmacokinetic studies in healthy subjects were collected and required analysis. As it has been previously reported that rabeprazole is unstable in human serum [4], a fast and efficient HPLC method for rabeprazole and its thio-ether metabolite was needed.

A previous HPLC assay for rabeprazole and rabeprazole thio-ether has been published [5]; however, this assay did not meet the fast analysis requirements to accommodate the samples in our study, because the thio-ether retention time was 19.4 minutes.

This was also the case with a published gradient HPLC system: the run time of rabeprazole appeared longer than 25 minutes [6, 7]. Two recent papers investigated the use of solid-phase extraction for rabeprazole. In one paper, the metabolite rabeprazole thio-ether was not determined [8] and in the other, the run time of rabeprazole thio-ether appeared to be longer than 50 minutes [9]. The objective of the present study was to develop a fast and efficient HPLC method for the determination of rabeprazole and its metabolite rabeprazole thio-ether in human serum samples.
Determination of rabeprazole and metabolite in human serum using high-speed HPLC

METHODS

Chemicals and reagents
Acetonitrile and methanol were both from the high-grade Lichrosolv range. Phosphoric acid, sodium dihydrogen phosphate, sodium hydroxide, potassium hydroxide, diethylamine (DEA), heptane/isoamylethanol and tertiary butylmethylether (t-BME) were supplied by Merck (Darmstadt, Germany), all pro-analysis quality. Dichloromethane, HPLC grade, was obtained from Rathburn (Walkerburn, Scotland). Phosphate buffer (pH 7.2, 0.05 M) was prepared according to the European Pharmacopoeia (5th edition). Purified water was obtained from a reversed osmosis system from Christ (Aesch, Switzerland). Blank calf serum was obtained from Invitrogen (Groningen, the Netherlands). Rabeprazole (lot number 11041501) and rabeprazole thioether (lot number 18040610) were kindly supplied by Eisai (Tokyo, Japan) and lansoprazole (lot number HB261) by Hoechst Marion Roussel (Hoevelaken, the Netherlands).

Instrumentation and chromatographic conditions
The HPLC system that was used consisted of a quaternary pump, an autosampler, a thermostated column compartment set at 40°C and a diode array detector coupled with Chemstation software from Agilent Technologies (Waldbronn, Germany). The separation of rabeprazole was carried out on a Zorbax Eclipse XBD C18 rapid resolution column (4.6 mm x 30 mm, 3.5 μm particle size) from Agilent Technologies (Waldbronn, Germany). The wavelength for detection was 284 nm. The mobile phase consisted of a mixture of 650 mL water and 300 μL phosphoric acid, set at pH 7.0 with 10% potassium hydroxide, followed by addition of 350 mL acetonitrile (water-acetonitrile ratio: 65:35, phosphoric acid: 4.45 mM). Elution was performed in an isocratic mode (flow set at 2 mL/min). The analyses were carried out at an ambient temperature of 20°C.

Preparation of standards and controls
Rabeprazole and rabeprazole thio-ether stock solutions (10 mg/50 mL methanol with 0.1% DEA) were diluted to working solutions containing 10 ng/μL rabeprazole and 20 ng/μL rabeprazole thio-ether in 0.1% DEA in methanol. A calibration curve of 0.015-1.4 mg/L was made for rabeprazole by adding aliquots of the working solution to 1.0 mL of blank calf serum, diluted 10:1 with 1% DEA in water. Additionally, aliquots of rabeprazole thio-ether were added in the same manner to obtain a calibration curve of 0.025-1 mg/L.

In order to stabilise the samples to prevent degradation, 0.1% DEA in methanol was added to the samples for the calibration curve, so each sample contained 25 μL 0.1% DEA in methanol. Standard curves were constructed by non-weighted linear regression.

To prepare quality control samples, 1.0 mL blank calf serum, diluted 10:1 with 1% DEA in water, was spiked with three different concentrations of independent working solutions in order to contain 0.015 (low), 0.25 (medium), and 0.7 (high) mg/L rabeprazole and 0.026 (low), 0.52 (medium) and 1.0 (high) mg/L rabeprazole thio-ether.

Sample preparation
To prepare the samples, aliquots of 1.0 mL of serum were mixed with 100 μL of the internal standard lansoprazole, 0.5 mL phosphate buffer was added, followed by 5 mL of t-BME. Samples were shaken (200/minute) for 10 minutes and centrifuged for five minutes (2,550 g). The organic layer was transferred into a disposable 12 mL glass tube and evaporated to dryness at 25°C under a stream of nitrogen. The dried analytes were reconstituted in 75 μL 0.1% DEA in mobile phase. Aliquots of 5 μL were injected into the HPLC system.
Assay validation
The precision (expressed as the percentage coefficient of variation, CV%) and accuracy (expressed as percentage bias) of the method described were assessed both within and between runs. The linearity, LLOQ, recovery and stability were also determined.

The acceptance criteria were set according to Shah et al. with minor modifications [10]. For precision, the acceptance criterion was set at a coefficient of variation (CV) of < 5%, for intra-assay CV the acceptance criteria were set at < 5% with a bias of < 15% for low control samples and of < 5% for medium and high control samples. For interassay, a CV of low control samples of < 20% with a bias of < 15% was accepted, and for medium and high control samples a CV of < 5% with a bias of < 5% was within the range of acceptance.

Linearity was determined with calibration standards prepared in duplicate. The LLOQ was calculated from the calibration curve by non-weighted linear regression. We defined LLOQ as the y-axis intercept plus 3.3 times the standard deviation and extrapolated this value towards x. In case the intercept was negative, we defined LLOQ as 10 times the standard deviation [11].

Serum concentration calibration curves were constructed by plotting the peak height ratios against the concentration of each drug or metabolite. The values obtained were analysed using analysis of variance (ANOVA). A correlation of at least 0.99 was desirable and the F-test for lack of fit (LOF) (one-sided, 95% confidence interval, CI) was applied. A critical LOF value of < 4.53 was within the range of acceptance.

Blank calf serum was used for most of the validation procedures for ethical reasons and also because of a lack of human serum. Rabeprazole, rabeprazole thio-ether and the internal standard showed identical behaviour in both calf serum and human serum, allowing part of the validation to be performed in calf serum.

Interference of drugs other than rabeprazole was not tested, because the healthy subjects that participated in the pharmacokinetic study were only included if they did not take any other drugs; this analysis was not used for any other purposes than for this pharmacokinetic study.

Recovery of rabeprazole from calf serum and from human serum was evaluated by comparing the mean peak responses of six quality control samples with mean peak responses of six plain standards of equivalent concentration. Recovery was defined as the percentage of the concentration in the 0.1% DEA in methanol solution determined in the sample. A recovery of > 70% was accepted, with a CV of < 5%.

The accuracy was evaluated by back-calculation and expressed as the percentage deviation between the amount found and the amount added to the concentrations examined. The acceptance criterion was set at < 5% deviation from the nominal value and < 5% deviation between human serum samples and control samples.

Auto-sampler stability of rabeprazole and its thio-ether in mobile phase was established by repeated analysis of a batch (low, medium and high) after 24 hours. All samples were considered acceptable where repeated samples differed by less than 20% for low control samples. For medium and high control samples, a difference of < 5% was within the range of acceptance. Stability and the effect of one freeze and thaw cycle were assessed in the quality control samples kept at −70°C. Stability of quality control samples (low, medium, high) was considered acceptable when analytical results from repeated samples differed by < 20% from initial samples.
Human pharmacokinetic study
The study was approved by the ethics review board of Haga Teaching Hospital (approval number 04.008) and written informed consent was obtained from each participant. Oral rabeprazole 20 mg was given to healthy volunteers after an overnight fast. Venous blood samples were collected in Vacutainer tubes at 0 (pre-dose), 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours after dosing. The tubes were centrifuged immediately at 2,550 g for 10 minutes, 100 μL of 0.1% DEA in water was added to 1 mL of the serum samples immediately after centrifuging, to make them more stable, and samples were stored at −70°C until analysis.

RESULTS AND DISCUSSION

Chromatographic conditions
For optimisation of chromatographic conditions, the pH was varied and set at pH 7.0. A decrease below pH 7 would have resulted in a lowering of the rabeprazole peak, and a setting above pH 7 would have resulted in a degradation of the HPLC column. The acetonitrile-water ratio was tested and more acetonitrile resulted in faster run times, but with poorer resolution. The optimal acetonitrile-water ratio appeared to be 35:65. This resulted in a run time of 1.5 minutes. The column temperature was also studied and an increase of the temperature up to 40°C resulted in an optimal peak shape with increasing peak height. The detection wave length was set at the maximum of 284 nm. Lower wave lengths were also tested; however, they resulted in substantial interference. The pH during the sample extraction was varied from pH 7 to pH 12 with buffer solutions. The optimum at which maximal recovery was achieved was reached with a phosphate buffer solution pH 7.2 (0.05 M). Comparison of dichloromethane, heptane/isoamylethanol and t-BME as extracting agent showed the best recovery with dichloromethane and t-BME, but t-BME was chosen because of its specific gravity. The capacity of bio-analysis was 20 samples per hour.

Recovery from calf serum and human serum
The recovery of rabeprazole and rabeprazole thio-ether from blank calf serum, under the conditions described for this assay, are given in Table 1. The recovery of the internal standard lansoprazole 0.3 mg/L was 97.8% (n = 6, CV = 0.8%). Six different human serum samples were spiked to a concentration of 0.252 mg/L for rabeprazole and 0.521 mg/L for rabeprazole thio-ether and were calculated on a standard curve based on calf serum. The mean concentrations found were 0.254 mg/L (n = 6, CV = 7.2%) and 0.511 mg/L (n = 6, CV = 1.5%). When compared with blank calf serum, the accuracy in human serum samples was 100.9% for rabeprazole and 98.1% for rabeprazole thio-ether. Representative chromatograms of rabeprazole and its metabolite are shown in Figure 2.
Figure 2 Chromatograms of blank human serum, lowest control sample and subject sample of rabeprazole and rabeprazole thio-ether

A Blank human serum sample, B Lowest control sample: rabeprazole 0.015 mg/L and rabeprazole thio-ether 0.026 mg/L, and C Subject: rabeprazole 0.45 mg/L and rabeprazole thio-ether 0.13 mg/L.
Calibration curve and LLOQ
The LLOQ for rabeprazole was 0.015 mg/L \((n = 6, \text{ CV} = 11.9\%)\), and for rabeprazole thio-ether 0.026 mg/L \((n = 6, \text{ CV} = 12.6\%)\). The calibration curve of rabeprazole resulted in a correlation coefficient of 0.9999 (range: 0.015-1.4 mg/L) with a LOF of 0.41. The calibration curve of rabeprazole thio-ether resulted in a correlation coefficient of 0.999 (range: 0.026-1.0 mg/L) with a LOF of 15.45. Because of the high LOF, the range of the calibration curve of rabeprazole thio-ether was set at 0.026-0.5 mg/L (recalculated LOF: 0.34).

Precision and accuracy
The precision and accuracy data from intra-day and interday analysis from three spiked concentrations of rabeprazole and rabeprazole thio-ether in calf serum are shown in Table 1. Regarding intra-day data, the CV of the lowest concentration of rabeprazole thio-ether did not meet the acceptance criterion of < 4%; however, the bias was within the range of acceptance (< 15%).

Table 1 Accuracy and precision of intra-day assay, inter-day assay and recovery of rabeprazole and rabeprazole thio-ether \((n = 6)\)

<table>
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<th>Compound</th>
<th>Added (mg/L)</th>
<th>Found mean</th>
<th>CV (%)</th>
<th>Bias (%)</th>
<th>Found mean</th>
<th>CV (%)</th>
<th>Bias (%)</th>
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<th>CV (%)</th>
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<td>11.6</td>
<td></td>
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<td>11.9</td>
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<tr>
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<td>8.0</td>
<td>0.0262</td>
<td>12.6</td>
<td>0.7</td>
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<td>3.7</td>
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<td>3.5</td>
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</tr>
</tbody>
</table>

Stability during processing and storage
Rabeprazole and its metabolites were shown to be unstable in serum samples without taking precautions. Samples stored at room temperature and at −70°C showed a rapid decomposition for rabeprazole and its metabolites (data not shown). For this reason, samples had to be stabilised using DEA, as has been published by Nakai et al. [5]. Unfortunately the mechanism of how DEA stabilises the samples is unknown. Addition of DEA guarantees stability in the freezer during storage. The quality control samples (low, medium, and high) in the auto sampler were stable for at least 24 hours and were within the range of acceptance (CV of < 5% with bias of < 5%). Results of one freeze-thaw cycle after six months of storage at −70°C showed a concentration of 100.2%, 101.6% and 99.3% respectively for low, medium and high rabeprazole quality control samples, and of 102.4%, 104.3% and 98.8% of rabeprazole thio-ether.
Pharmacokinetic analysis
Interim evaluation of the pharmacokinetic data of six homozygous extensive metabolizer volunteers showed median values of a $C_{\text{max}}$ of 0.26 mg/L, a $T_{\text{max}}$ of 3.55 hours, with a $t_{1/2}$ of 1.07 hours and an AUC of 362.8 ng x h/mL for rabeprazole, and a $C_{\text{max}}$ of 0.069 mg/L, a $T_{\text{max}}$ of 6.0 hours, with a $t_{1/2}$ of 3.11 hours and an AUC of 243.6 ng x h/mL for rabeprazole thio-ether.

Figure 3 shows a representative serum concentration versus time curve of rabeprazole and its metabolite in the serum of a healthy volunteer.

**Figure 3** Concentration-time curve of rabeprazole and rabeprazole thio-ether in a homozygous extensive metabolizer after intake of 20 mg of rabeprazole

CONCLUSION
The high speed HPLC method used at present proved to be applicable in this pharmacokinetic study. Quantification of rabeprazole and rabeprazole thio-ether by a high speed HPLC method is very fast (run time < 1.5 minutes), accurate and precise, and the method is appropriate for rapid determination of serum concentrations, especially when there is a large number of samples requiring analysis.
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Systematic review: the influence of CYP2C19 polymorphism on the acid-inhibitory effects of proton pump inhibitors

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Submitted
ABSTRACT

Aim
to conduct a systematic review on the influence of CYP2C19 polymorphisms on acid-suppressive therapy with proton pump inhibitors (PPIs).

Methods
Pubmed, Embase and Central were searched up to December 2009 for the indexed terms: “CYP2C19”, “proton pump inhibitors” or “esomeprazole / omeprazole / lansoprazole / pantoprazole / rabeprazole”. Studies were scored with a level of evidence and magnitude.

Results
Fourteen studies investigating esomeprazole 40 mg, lansoprazole 30 mg, omeprazole 10 and 20 mg, and rabeprazole 10, 20 and 40 mg were included. In ten studies Japanese subjects had been investigated, in two studies Chinese and in two studies Caucasians. The studies focused on intragastric pH and on the proportion of time or percentage during 24 hours with intragastric pH above 3.0 or 4.0. Evidence of CYP2C19 influence on these endpoints was significant for lansoprazole, omeprazole and rabeprazole between Asian homEMs and PMs, and between Asian hetEMs and PMs and for pantoprazole between Caucasian homEMs and hetEMs.

Conclusion
Influence of CYP2C19 polymorphism on therapy with lansoprazole, omeprazole and rabeprazole is significant between Asian homEMs and PMs and between Asian hetEMs and PMs and for pantoprazole between Caucasian homEMs and hetEMs. Considering the small prevalence of PMs in the Caucasian population, genotyping before start of PPI therapy is not useful. The rationale to increase the initial doses of PPIs for Caucasian subjects or to switch to a less CYP2C19-dependent PPI needs further research, especially in homEMs and RMs.
INTRODUCTION

Inhibition of gastric acid secretion is important for successful treatment of acid-related diseases. Patients with GERD experience (recurrence of) symptoms like chest pain and heartburn. These symptoms can cause clinical problems that negatively influence the quality of life [1, 2]. Proton pump inhibitors (PPIs) exert their effect through inhibition of acid production in the intragastric proton pumps, leading to elevation of intragastric pH. After entering the bloodstream, PPIs are metabolized by cytochrome P-450 enzymes in the liver. The main enzyme involved in the metabolism is CYP2C19. This enzyme shows functional genetic polymorphism. Studies that investigated the relationship between pharmacokinetics and dynamics of omeprazole have demonstrated that the acid inhibitory effect is related to the area under the concentration-time curve (AUC) of the drug. The AUC depends on a subject's CYP2C19 genotype [3]. The differences in metabolic capacity for PPIs related to CYP2C19 polymorphism were described by Chang and Ieiri [4, 5]. It was discovered that the drug mephenytoin could be used to calculate the metabolic ratio of a subject to predict a person's phenotype [6-8]. Subjects with a strongly decreased metabolic capacity were considered poor metabolizers (PM) and subjects without decreased metabolic capacity were considered extensive metabolizers (EM). Later on, phenotypes were correlated with genotypic variants of CYP2C19 by DNA analyses [4]. For CYP2C19, over 20 variants have been identified [9]. Homozygous extensive metabolizers (homEMs) have two wildtype alleles (*1/*1). The most common variants are *2, *3 and *17. CYP2C19*2 and *3 are associated with decreased enzymatic activity, resulting in either heterozygous extensive metabolizers (hetEMs with *1/*2 or *1/*3 genotype) or poor metabolizers (*2/*2, *2/*3 or *3/*3 genotype). CYP2C19*17 is associated with increased enzymatic activity, resulting in homozygous rapid metabolizers (homRM, *17/*17) or heterozygous rapid metabolizers (hetRM, *1/*17 or *2/*17). The frequency of the variant alleles *2 and *3 is much higher in Asian populations than in European, African, South-American and Australian populations [10-17]. In contrast to *2 and *3 variants, the *17 variant is mainly found in Caucasians with an allele frequency of 17 to 20% [9, 18, 19]. Success or failure of PPI therapy is thought to be related to the CYP2C19 genotype, as this influences the systemic availability and clearance of the drug and thus the AUC and acid suppressive effect. In theory, this would imply that RMs and homEMs require higher PPI doses than hetEMs and PMs [20]. For this reason, it has been suggested to determine a subject's genotype before starting PPI therapy [21, 22]. However, the high efficacy and the excellent safety profile of these drugs together with the large variation amongst populations in the prevalence of the various genotypes do not support this advice. Considering this suggestion, we conducted a systematic review focusing on the influence of CYP2C19 *2, *3 and *17 variants on the acid-suppressive effects of PPIs.
METHODS

Pubmed, Embase and Central (the database from Cochrane) were searched up to December 2009 for the indexed terms: “CYP2C19”, “proton pump inhibitors” or “esomeprazole / omeprazole / lansoprazole / pantoprazole / rabeprazole”. Published studies evaluating the influence of CYP2C19 genotype status (RMs, homEMS, hetEMs and PMs analyzed separately) on the acid-inhibitory effects of orally administered PPIs (percentage of time with pH above the threshold 3 or 4 during 24 hours and mean (or median) 24-hour intragastric pH) were included. Only studies that investigated H. pylori-negative subjects were included. Endpoints were rated for evidence and magnitude according to the rating system in Table 1 [23]:

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The magnitude of the effect on the clinical outcome was also rated according to the scoring method used by the Royal Dutch Association for the Advancement of Pharmacy [adapted with minor modifications from [24], Table 2]. The endpoints were percentage of time (or hours) with pH above 3 or 4 and intragastric pH in healthy volunteers. Studies were separately rated by investigators NH and AG and checked by DT.

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RESULTS

The search strategy yielded 450 abstracts, of which 32 were relevant to the review topic and subsequently reviewed. Following evaluation of the full text papers nineteen of them were rejected because the results of homEMs, hetEMs, RMs and PMs were not analyzed separately. Thirteen studies and one abstract met the inclusion criteria were included in the final analysis (Table 1). Ten of them had investigated Japanese subjects, two studies had investigated Chinese Han subjects and the remaining two studies had been performed in Caucasian subjects. The mean sample size was 17 (range 15-20) subjects. No information about gender was provided in one study [25], six studies investigated male subjects [26-31] and seven studies investigated male and female subjects [21, 32-37]. Median (or mean) 24-hour intragastric pH was monitored in 8 studies [21, 25, 27, 28, 32, 33, 36, 37].

Ten studies focused on intragastric pH with hours or percentage of time above threshold pH 3 or 4 [25, 26, 28-35]. Four of these studies investigated both parameters [25, 28, 32, 33]. The threshold was set at pH 4 in all studies, with the exception of one study (pH 3) [25].

In all studies, CYP2C19*2 and/or *3 variants were studied. In the one study and one abstract that investigated *17 variants [32, 33], the number of subjects with these variants was too small for statistical analysis.

For each PPI, the reviewed studies with their level of evidence and magnitude are shown in Table 3.

The influence of CYP2C19 was most frequently investigated after administration of rabeprazole (Table 3E). After a single dose of 10 mg and 20 mg of rabeprazole a small, non-significant, difference in % time pH > 3 between homEMs and PMs and between hetEMs and PMs was observed (level 3A) [25]. Another study showed a significant difference in % time pH > 4 between homEMs and hetEMs after a single dose of 20 mg (level 4B) [33]. This difference decreased after repeated administration (level 4A).

Three other studies investigated repeated administration of 20 mg rabeprazole [26, 29, 30]. The influence of CYP2C19 was evident between homEMs and PMs in two studies (both level 4C) [26, 30]. After repeated administration with a low dose of rabeprazole of 10 mg no significant difference was found between genotypes (level 4A and 3A) [29, 30]. In addition, no difference was observed after 10 mg of rabeprazole twice daily [30]. Repeated administration of 40 mg of rabeprazole resulted in significant differences between homEM and PMs and between hetEMs and PMs (level 4C) [26].

Significant differences in 24 hour intragastric pH after a single dose of rabeprazole 10 mg were observed between homEMs and PMs and between hetEMs and PMs (level 3B) [25]. Data from four studies that investigated rabeprazole 20 mg after a single dose showed a difference between genotypes. This difference was significant between Japanese homEMs and PMs in one study (level 4B) [36] and between homEMs and PMs and hetEMs and PMs in another study (level 3B) [25]. No significant differences in intragastric pH between genotypes were observed after repeated dosing of 20 mg of rabeprazole (level 4A), with the exception of one study in Caucasians (homEMs vs. hetEMs, level 1B) [33].
With omeprazole (Table 3C), one study showed a significant difference in % time pH > 4 between homEMs and PMs and between hetEMs and PMs after repeated administration of 10 mg (level 3B) [31]. Significant differences between the three genotypes were also shown after a single dose of omeprazole 20 mg (level 3B) [28]. In this study, repeated administration showed no difference between homEMs and hetEMs (level 3A), but, in line with the previous study, differences between homEMs and PMs (level 3B) and between hetEMs and PMs (level 3B) remained significant. The latter findings were also confirmed in another study (level 3C) [31].

Studies that investigated the intragastric pH showed significant differences between the genotypes after a single dose of omeprazole 20 mg (level 4B) [21, 28, 36]. After repeated administration, consistent differences were observed between homEMs and PMs (level 4B) [28, 36]. A study in Japanese subjects showed a significant difference between homEMs and hetEMs (level 4B) [36]. This was not observed in Chinese Han subjects (level 3A) [28].

No studies were included that investigated the influence of CYP2C19 polymorphism after single administration of lansoprazole (Table 3B). Repeated administration of lansoprazole was studied in two dosages: 30 mg once daily and 30 mg twice daily. The influence of CYP2C19 on % time pH > 4 after 30 mg once daily is consistent. A significant difference between the three genotypes was observed in two studies (level 4B/3C) [29, 35]. This difference was also observed after administration of 30 mg twice daily (level 4C, homEMs vs. PMs and hetEMs vs. PMs) [34]. The intragastric pH was monitored in one study. After repeated administration of lansoprazole 30 mg a significant difference was shown between homEMs and PMs (level 4B) [37].

The evidence for influence of CYP219 on esomeprazole 40 mg after single and repeated dosing is inconsistent for both % time pH > 4 and intragastric pH (Table 3A). One study showed no influence between homEMs and hetEM (level 4A) [32], while a second study showed a significant difference between genotypes (level 1B) [33].

One study showed a significant difference in % time pH > 4 and intragastric pH between homEMs and hetEMs after single and multiple dosing with pantoprazole 40 mg (level 4B) (Table 3D) [32].
Table 3A, B, C, D, E: PPIs and reviewed studies with level of evidence and magnitude

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- pH > 4: (percentage of) time with intragastric pH > 4, pH IG: mean or median 24-hour intragastric pH, RD: randomized, PC: placebo-controlled, DB: double-blind, CO: cross-over, IB: investigator blinded, Cau: Caucasian, J: Japanese, C: Chinese, n: number, -: no data, NS: not significant, N: nocturnal, D: daytime. All subjects were healthy and H. pylori negative.

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Systematic review: the influence of CYP2C19 polymorphism on the acid-inhibitory effects of proton pump inhibitors
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<td>hetEM vs. PM</td>
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DISCUSSION

This systematic review shows that there is evidence for the influence of CYP2C19 genotype on the acid-inhibitory effects of all PPIs. In the included studies, PPIs have been studied in different doses and after different durations of therapy. Therefore, we analyzed all PPI doses separately and as a consequence, no meta-analysis could be performed.

The evidence regarding the influence of CYP2C19*2 and *3 variants is consistent for higher PPI doses after both single and repeated administration. This evidence is especially observed between the homEM and PM phenotypes and between the hetEM and PM phenotypes in Asian subjects. There is a lack of data for homEMs and hetEMs in general and for RMs from Caucasian origin.

The influence of CYP2C19 after a single dose is of clinical significance, since many patients use PPIs on an on-demand basis [38]. Both single and repeated dosing were therefore included in this review. There were more studies on repeated dosing included, however it seems that influence of CYP2C19 is irrespective of single or multiple administration.

Regarding the lower doses, rabeprazole 10 mg seems only to be influenced by CYP2C19 genotype after a single dose, while omeprazole 10 mg only seems to be influenced after repeated administration. The lower PPI doses might show a smaller genotype-dose effect than the higher doses. This could be caused by the larger variability in response shown at lower doses, overruling any genotypic influence [3]. No data are available for esomeprazole 20 mg, lansoprazole 15 mg, and pantoprazole 20 mg.

Only studies that investigated CYP2C19*2 and *3 variants could be included in this review. The majority of the studies has been performed in Asian subjects. The genotypic disposition in the Asian population differs from that in the Caucasian population. Not only the prevalence of *2 and *3 variants is different, also the prevalence of the *17 variants varies. The prevalence of *17 variants in Caucasian subjects is about 32% [39], while its prevalence in Japanese subjects is only 1% [40]. Data about *17 variants are limited, but so far it has been shown that *17 variants are associated with increased metabolism of omeprazole, resulting in (ultra)rapid metabolizers [18, 41]. One retrospective study investigated the influence of CYP2C19*17 variants on PPIs [42]. It was shown that Caucasian subjects with *1/*17 genotype need stronger acid-suppression therapy, especially after the first days of treatment or with on-demand therapy. Two prospective studies confirmed the lower acid-inhibitory effect in subjects with *1/*17 genotype, but their number was too small for statistical analysis [32, 33]. Larger prospective studies that are adequately powered for CYP2C19*17 are warranted.

Besides the differences in prevalence of CYP2C19 variants between the Asian and the Caucasian population, studies have demonstrated that Caucasian EMs have a higher clearance of omeprazole than Chinese and Korean EMs [43, 44]. A plausible hypothesis for this difference in clearance could be the presence of variant genes of CYP2C19, like *17 or yet undiscovered variants with higher metabolic capacity in Caucasians. Another hypothesis for the difference in clearance could be a different capacity of CYP3A4, the other main enzyme involved in PPI metabolism, in Caucasians compared with Asian subjects [45].
Recently, it has been suggested to genotype all patients before starting a PPI [46]. Based on our review, the impact of CYP2C19 variants seems of clinical importance between Asian homEMs and PMs and between Asian hetEMs and PMs, using omeprazole, lansoprazole or rabeprazole. These results would imply that only the Asian population with about 20% of PMs would benefit from genotyping. Data from Caucasians show a significant difference between homEMs and hetEMs after single and repeated administration of pantoprazole and rabeprazole. There are no data of pantoprazole or esomeprazole in Asian subjects. For the Caucasian population, with a majority of rapid and extensive metabolizers, administration of a PPI with the least CYP2C19 involvement (e.g. esomeprazole) or an increase of the initial doses of PPIs, would seem a more rational approach than genotyping. These approaches need further research.

In summary, all PPIs are more or less influenced by CYP2C19 polymorphism, especially after repeated administration with higher doses. This review shows that the clinical relevance of CYP2C19 polymorphism in the treatment of acid-related diseases has to be evaluated separately for each PPI, for each race and for each genotype. Based on this systematic review, the order of CYP2C19 influence between homEMs, hetEMs and PMs for the higher PPI doses is: rabeprazole 20 mg > lansoprazole 30 mg > omeprazole 20 mg > pantoprazole 40 mg > esomeprazole 40 mg. For the lower doses, the order is: omeprazole 10 mg > rabeprazole 10 mg.

Considering the small prevalence of PMs in the Caucasian population, genotyping before start of PPI therapy is not useful. The rationale to increase the initial doses of PPI for Caucasian subjects or to switch to a less CYP2C19-dependent PPI needs further research, especially in homEMs and RMs.
REFERENCES


General discussion
INTRODUCTION

Proton pump inhibitors (PPIs) are the cornerstone in the treatment of acid-related diseases. PPIs suppress gastric acid secretion by specific inhibition of the H⁺/K⁺-ATPase in the gastric parietal cell. This results in inhibition of the acid secretion, followed by elevation of the intragastric pH [1]. Since the introduction of PPIs in the ‘80s, their use is still increasing. In the management of acid-related diseases, PPIs are generally prescribed in a once daily fixed dose regimen, implying a ‘one dose fits all’ strategy. Although all PPIs are effective acid-suppressive drugs, studies have shown a large inter- and intra-individual variability in response to PPIs [2, 3]. This variability in response to PPIs may lead to an unpredictable effect of the therapy. Three pharmacological parameters may attribute to the variability in response to PPIs: pharmacogenetics, pharmacokinetics and pharmacodynamics. This thesis investigated the role of pharmacogenetics on pharmacokinetics and on pharmacodynamics for better understanding and improvement of therapy with PPIs.
PHARMACOGENETICS AND PPIs

One of the aims of this thesis was to investigate the impact of pharmacogenetics on the acid-inhibitory effects of PPIs. CYP2C19 and CYP3A4 are the main enzymes responsible for the metabolism of PPIs. Of these two, genetic variation of CYP2C19 is associated with variation of the clinical effect of PPIs [4]. Most studies investigating the influence of CYP2C19 variants on the pharmacokinetics and pharmacodynamics of PPIs have been performed in selected groups of non-Caucasian subjects. No information about the influence of CYP2C19 genotype on the pharmacodynamics of pantoprazole was available and most studies did not have a comparable design. We therefore assessed the impact of CYP2C19 on the kinetics and dynamics of esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole in Caucasian populations (Chapter 4, 5 and 6, in an identical design) and we systemically reviewed all studies about CYP2C19 and PPIs (Chapter 8).

Table 1 shows an overview of the prevalence and clinical effects of CYP2C19 variants in different populations [5-11]. Subjects with *1/*1 (wildtype/wildtype) genotype are considered as homozygous extensive metabolizers (homEMs) associated with normal pharmacokinetics and pharmacodynamics. Their prevalence has been shown to range from 35% in Japanese to 39% in Caucasians, and to 52% in Chinese [6, 8, 11]. The *2 and *3 variants are held responsible for a decreased metabolism of PPIs resulting in heterozygous extensive metabolizers (hetEMs, *1/*2 or *1/*3 variants) and in poor metabolizers (PMs, *2/*2, *3/*3 or *2/*3 variants). In Eurasia, an increase in the prevalence of *2 and *3 variants has been observed from West to East. In the Caucasian population about 25% has *1/*2 genotype and 3% has *2/*2 genotype [9]. In the Chinese population, about 40% has *1/*2 or *1/*3 and 12% has *2/*2, *2/*3 or *3/*3 genotype [6]. In the Japanese, about 55% has *1/*2 or *1/*3 and 20% has *2/*2, *2/*3 or *3/*3 genotype [7, 8]. In contrast to *2 and *3 variants, the *17 variant is associated with an increased metabolic rate (phenotype: (ultra)rapid metabolizers ((U)RM)) and may lead to under treatment with drugs metabolized by this enzyme in subjects carrying one or two alleles with this variant [12]. The prevalence of CYP2C19*17 mutations among populations has been shown to be the opposite of the *2 and *3 variants. About 27% of the Caucasian population has *1/*17 or *17/*17 genotype compared to 1% of the Chinese and 3% of the Japanese population [6, 8, 10]. The prevalence of CYP2C19*17 variant genotypes (*1/*17, *2/*17 or *17/*17) in the Dutch population (34%) was comparable to that of other Caucasian subgroups [11, 13]. Apart from *17 and *2 mutations, no *3, *4, *5 or *6 variants have been found in our Dutch study population (Chapter 3) [11].
Table 1 Prevalence and clinical effects of CYP2C19 genotypes in different populations

<table>
<thead>
<tr>
<th>CYP2C19 variant</th>
<th>Possible genotypes</th>
<th>Genotype prevalence in:</th>
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<tr>
<td></td>
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<td>Caucasians (Dutch) (%)</td>
<td>Asians (Chinese) (%)</td>
<td>Asians (Japanese) (%)</td>
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<td>*17</td>
<td>*17/*17</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
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<td></td>
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<td>1.1</td>
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<tr>
<td></td>
<td>*2/*17</td>
<td>8</td>
<td>0</td>
<td>1.5</td>
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<td></td>
<td>*3/*17</td>
<td>0</td>
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<td>*1</td>
<td>*1/*1</td>
<td>39</td>
<td>52.1</td>
<td>35.5</td>
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<td>*2</td>
<td>*1/*2</td>
<td>25</td>
<td>33.7</td>
<td>40.2</td>
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<td></td>
<td>*2/*2</td>
<td>2.9 (1.5)</td>
<td>9.2</td>
<td>9.7</td>
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<tr>
<td>*3</td>
<td>*1/*3</td>
<td>0.2 (&lt; 0.3)</td>
<td>6.1</td>
<td>14.7</td>
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<tr>
<td></td>
<td>*2/*3</td>
<td>0</td>
<td>2.8</td>
<td>7.4</td>
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<td>*3/*3</td>
<td>0</td>
<td>0</td>
<td>2.4</td>
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\(^1\): Since data about the prevalence of genotypes from different references are combined, the total percentage of 100% can be exceeded.
\(^2\): area under the concentration time curve
\(^3\): Frequencies of *2/*17 and *3/*17 were together 1.5%.
\(^^\): unknown.

Although prospective studies are warranted, our data clearly have shown that knowledge about the studied populations (Which race? Which prevalence of variants?) is of cardinal importance in interpreting data from clinical studies on acid suppression with PPIs. Extrapolation to a different population is only possible with knowledge of the prevalence of its genotypes and phenotypes (URM, RM, homEM, hetEM or PM). For example, a study that has investigated the efficacy of PPIs in Asian subjects (mainly hetEMs and PMs) leads to a much better response to PPIs than a study that has been performed in Caucasians (mainly homEMs and RMs) when the same dose is used. In more detail, a comparison between the pharmacodynamic response in Asians and in Caucasians can only be made if studies have investigated the different genotypes separately. In general, data from clinical studies that have investigated drugs that are metabolized by CYP2C19 cannot be extrapolated from one population to another population. For clinicians prescribing drugs that are metabolized by CYP2C19, the differences in response caused by CYP2C19 polymorphism warrant knowledge of genetic variants in their particular patient populations.
We have shown for the first time (Chapter 4) that Caucasian subjects with *1/*1 and *1/*17 genotype need stronger acid-suppression therapy than subjects with *1/*2 genotype, especially during the first days of treatment or with on-demand therapy [12]. This study investigated healthy volunteers with different genotypes (*1/*1, *1/*17, *1/*2). Their intragastric pH data at day 1 and day 5 of oral administration of four different PPIs (lansoprazole 15 mg, omeprazole 10 mg, omeprazole 20 mg and pantoprazole 40 mg) were compared to their baseline pH data (day 0). It was observed that *1/*17 genotype did not show significant acid-inhibition after administration of a single dose of omeprazole 10 mg, omeprazole 20 mg and lansoprazole 15 mg and after repeated administration of omeprazole 10 mg and lansoprazole 15 mg. Subjects with *1/*17 genotype did not show significant acid-inhibition after a single dose of omeprazole 20 mg and pantoprazole 40 mg. Subjects with *1/*2 genotype showed significant acid-inhibition after single and repeated administration of lansoprazole 15 mg and omeprazole 10 mg.

The influence of CYP2C19 genotype on the clinical effects of oral esomeprazole, pantoprazole and rabeprazole was prospectively investigated in two randomized clinical studies in healthy *H. pylori*-negative Caucasian subjects [14, 15]. One study (Chapter 5) investigated esomeprazole 40 mg and pantoprazole 40 mg after single and repeated administration. It showed that pantoprazole was influenced by CYP2C19 genotype. A significant difference in acid-inhibition (percentage of time with pH > 4 and median 24-h intragastric pH) was observed at day 1 and at day 5. This was accompanied by a significant difference between *1/*1 and *1/*2 genotype in the pharmacokinetics (area under the serum concentration vs. time curve (AUC)). In contrast, no significant difference in the acid-inhibitory effects and in the pharmacokinetics was observed between *1/*1 and *1/*2 genotypes after administration of esomeprazole [14].

Data from a study that investigated esomeprazole 40 mg and rabeprazole 20 mg after single and repeated administration are shown in Chapter 6 [15]. This study showed that differences in acid-inhibition between *1/*1 and *1/*2 genotypes were significant for both esomeprazole and rabeprazole.

We have investigated the difference between *1/*1 and *1/*2 genotypes on the acid-inhibitory effects of esomeprazole 40 mg in two separate studies (Chapter 5 and 6). Since our two studies were identical in design, the conflicting results of the influence of CYP2C19 after administration of esomeprazole may be caused by small differences in acid-suppressive response between subjects with *1/*1 and *1/*2 genotypes. Although the studies were powered to detect significant differences between *1/*1 and *1/*2 genotype, a type II error could have occurred. A larger prospective study, with also a *1/*17 group included, is warranted. Data from other studies that investigated the acid suppressive effects esomeprazole did not show a genotypic influence of CYP2C19 [16, 17]. These studies however had a different design or objective than our studies.

One open, randomized crossover study was designed to evaluate the effect of single and repeated administration of esomeprazole 40 mg on intragastric pH in healthy Chinese extensive metabolizers (EMs) (no difference was made between homEMs and hetEMs) compared with PMs. On genotype analysis, 28 of the subjects were EM and eight were PM. Those who were PM tended to have a higher, albeit not statistically significant, percentage of time with intragastric pH > 4 and median 24-h intragastric pH than those who were EM [16]. In another study, it was tested whether esomeprazole-induced healing of GERD was related to CYP2C19 genotype. The results showed that the frequency distribution of CYP2C19 genotypes was not different between patients with complete and incomplete healing [17].
In a systematic review (Chapter 8), we showed that the PPIs esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole are more or less influenced by CYP2C19 polymorphism, especially at higher doses [18]. The clinical relevance of CYP2C19 polymorphism in the treatment of acid-related diseases needs to be evaluated separately for each PPI, for each race and for each genotype. In more detail, the influence of CYP2C19 polymorphism on therapy with lansoprazole, omeprazole and rabeprazole was significant between Asian homEMs and PMs and between Asian hetEMs and PMs. Considering the low prevalence of PMs in the Caucasian population, genotyping before start of PPI therapy is not useful for Caucasians.

In line with our previous studies (Chapter 4, 5 and 6), the rationale to increase the initial doses of PPIs for Caucasian subjects or to switch to a less CYP2C19-dependent PPI needs further research, especially in homEMs and (U)RMs [12, 14, 15]. And in the perspective of our findings, the ‘one dose fits all’ strategy for PPIs needs to be changed into ‘individualized therapy’.

**PHARMACOKINETICS AND PPIs**

In order to be able to study the pharmacokinetics in our studies, esomeprazole, pantoprazole and rabeprazole serum concentration levels were analyzed by means of liquid chromatography techniques (HPLC). Esomeprazole and pantoprazole could be analyzed by existing methods [19]. For rabeprazole, no analysis was available in our laboratory. The analysis of rabeprazole in human serum was complicated by the unstable properties of the drug and its long run time during analysis. We therefore developed and validated a fast and efficient analysis for the determination of rabeprazole and its metabolite in human serum (Chapter 7), that was suitable for the analysis of the serum concentration levels during our pharmacokinetic study. The measured serum concentrations were used to calculate pharmacokinetic parameters, like maximal serum drug concentration (C\text{max}), clearance (CL) and the AUC.

PPIs have shown a poor correlation between the C\text{max} and the degree of acid suppression. The maximal serum drug concentration varied widely depending on the rate of passage in the gastrointestinal tract, release of drug and the intraduodenal pH. However, AUC correlated well with acid suppression for both esomeprazole and omeprazole [20, 21]. After repeated administration of omeprazole or esomeprazole, the C\text{max} and AUC increased in a nonlinear fashion [14, 22], which is due to decreased first-pass elimination and decreased systemic clearance. An explanation for these effects is auto-inhibition of CYP2C19 [4]. After repeated administration of rabeprazole and pantoprazole no increase in AUC was observed, confirming the absence of auto-inhibition of CYP2C19 of these PPIs [14, 15]. Besides auto-inhibition of CYP2C19, administration of esomeprazole resulted in higher AUC values than administration of racemic omeprazole. This was caused by a lower metabolic rate of esomeprazole compared with R-omeprazole [14, 22].

The pharmacokinetic-pharmacodynamic (PK-PD) data from our studies with esomeprazole confirmed previous data that an increase in AUC results in an increase in the percentage of time with pH > 4 (Chapter 5 and 6). With both pantoprazole and rabeprazole, also a PK-PD correlation was observed; however, their maximal acid-inhibitory effect was markedly lower than that from esomeprazole. This observation raised the question whether pantoprazole and rabeprazole show a maximum acid-inhibitory effect after administration of 40 mg, respectively 20 mg. For pantoprazole data from other studies not only demonstrated that pantoprazole showed a linear dose-effect relationship in the range of 10–40 mg once daily [23], but also showed that increasing the dose above 40 mg did not lead to an increased median pH elevation [24-27]. These data supported our hypothesis that for pantoprazole the acid inhibitory effect is maximized to 70% of percentage of time with pH > 4. For rabeprazole, our findings could not be confirmed by other data.
PHARMACODYNAMICS AND PPIs

The ‘gold standard’ of measuring the acid-inhibitory effects of PPIs is 24-hour intragastric pH monitoring. With continuous intragastric pH monitoring, two parameters are calculated. The first one is the median pH value over predefined time periods (median intragastric pH). The second parameter is the cumulative percentage of time that intragastric pH value is above pH threshold 4 (% time > pH 4). Maintenance of pH > 4 is an important objective in management of GERD. In GERD patients healing of reflux oesophagitis correlates directly with the percentage of time that intragastric pH is above pH 4 in a 24-h period and this is considered the key to effective management of reflux disease [28]. When studying the effect of a single PPI, a baseline measurement is necessary in order to observe a (significant) change in intragastric pH. A baseline measurement is also necessary for investigating the impact of genotypes on the acid-suppression of PPIs, as has previously been shown by our group [12]. Furthermore, baseline data can be used to calculate the amount of responders and non-responders to PPIs. This ‘response parameter’ was introduced by our group, because of a lack of a definition of response in pH-metry studies. To determine the net response to the study drug, the cumulative percentage of time with pH above threshold 4 at baseline was subtracted from the cumulative percentage of time with pH above threshold 4 at day 1 and at day 5 (or 6) for each subject. This gain is represented as Δ percentage of time with intragastric pH > 4. We defined individuals with a Δ of ≥ 10% as responders and individuals with a Δ of < 10% as nonresponders. The cut-off value between response and nonresponse was set at 10% because of the accuracy of the technique of intragastric pH-monitoring and the variability in 24-h intragastric acidity [29]. This new parameter has now been successfully used in three of our studies. It has been instrumental in determining differences in response to the different PPIs (Chapter 4, 5 and 6).

The acid-inhibitory effects between PPIs can be studied by comparing the PPIs in a cross-over design. In cross-over studies, intra-individual comparisons may be affected by the H. pylori status of the subjects. H. pylori exaggerates the acid suppressive effects of PPIs [30-33]. During treatment with these drugs, H. pylori-positive subjects consequently have a higher intragastric pH than H. pylori-negative subjects. This can be explained by the interaction between H. pylori colonization and acid production. H. pylori causes chronic gastritis in almost all subjects colonized with this bacterium. In subjects with normal acid production, gastritis is largely confined to the gastric antrum. There is general agreement that acid-suppressive therapy changes the usually antral-predominant gastritis to one that is corpus-predominant by simultaneous changes in the colonization pattern of H. pylori [34]. As such treatment with antisecretory agents may alter the pattern of H. pylori infection, introducing a carry-over effect in cross-over studies with subsets of H. pylori-positive subjects. In order to exclude this carry-over phenomenon, we only included H. pylori-negative subjects in our studies.

Although there are data about the differences in acid-inhibition between esomeprazole 40 mg, pantoprazole 40 mg and rabeprazole 20 mg, most studies did not report pharmacodynamics after both single dose administration (day 1, 24 h after administration) and during steady state (day 5, 120 h after administration). Even so, not many studies investigated the speed of onset of PPIs. This is clinically relevant as many patients nowadays use PPIs on a non-continuous basis [35]. Short intermittent treatment or on-demand therapy with a PPI requires an agent that has a rapid and sustained onset of action after a single dose.
Compared with the other PPIs, rabeprazole is less dependent on low pH for conversion to its active form owing to its higher pKa (5; the other proton pump inhibitors have a pKa ~4); therefore, rabeprazole undergoes rapid activation over a wider pH range. It has been suggested that because of its pKa characteristics, rabeprazole should produce a more rapid onset of acid-inhibition than the other PPIs [36-38]. Based on this information, the primary objective of our prospective studies was to compare the acid-inhibitory effects of PPIs at 4, 24 and 120 h after oral administration in a Caucasian population of H. pylori-negative subjects with known CYP2C19 genotype. Esomeprazole, pantoprazole and rabeprazole were compared in the dosages that are registered for the initial treatment of GERD [39-41]. The results from the first study showed that esomeprazole 40 mg provided faster and superior acid-inhibition than pantoprazole 40 mg after single and repeated administration [14]. The results from the second study showed that esomeprazole 40 mg provided superior acid-inhibition than rabeprazole 20 mg after single and repeated administration. Acid-inhibition with esomeprazole was faster, although not significant, than with rabeprazole [15]. The faster mode of action of esomeprazole was also observed by others [16, 42].

REBOUND ACID HYPERSECRETION (RAHS)

Serious questions have been raised whether cessation of PPI therapy results in RAHS. With the introduction of stronger acting PPIs, like esomeprazole, these questions needed to be answered. Many guidelines and publications mentioned RAHS as a significant side-effect in the prescription of PPIs, especially for general practitioners [43-45]. In this perspective, we conducted a systematic review of literature about RAHS after cessation of PPI therapy (Chapter 2). Only a small number of studies could be reviewed and the included studies were heterogenic in design, methods and outcome. There was some evidence from uncontrolled trials for an increased capacity to secrete acid in H. pylori-negative subjects after 8 weeks of treatment. Since the publication of our review, one other trial investigating RAHS has been published [46]. The design of this trial was randomized, double-blind and placebo-controlled. Healthy volunteers were randomized to 12 weeks of placebo or 8 weeks of esomeprazole 40 mg/d followed by 4 weeks with placebo. The Gastrointestinal Symptom Rating Scale (GSRS) was filled out weekly. The results showed that PPI therapy for 8 weeks induced acid-related symptoms in healthy volunteers after withdrawal. Although this study was placebo-controlled, remarks can be made by the design and results of the study. The investigators used a symptom rating scale. Such a scale is a surrogate parameter and could introduce a bias and the outcome cannot be linked to intragastric pH data. Furthermore, the study was performed in healthy subjects. This ruled out any influence of gastro-intestinal disease on the occurrence of RAHS in this study and limited extrapolation of the results to patients. Until now there is still no strong evidence that RAHS is clinically relevant, but because of the uncertainty and conflicting data, the potential of RAHS needs to be considered in particular in patients who have been treated with a PPI for longer duration and who previously experienced a rapid recurrence of symptoms after withdrawal of PPI treatment.
INDIVIDUALISATION OF PPI THERAPY IN CAUCASIANS: A PROPOSAL OF A STEPWISE APPROACH

The results with regard to the pharmacogenetics, kinetics and dynamics of PPIs provide a basis for a proposal for an individualised dosing regimen. This proposal is presented in figure 1. This individualised stepwise dosing regimen is designed to be clinically feasible. It is based on the rationale to increase the initial doses of PPIs for Caucasian subjects (consisting of 64% rapid metabolizers) and to switch to the PPI that is the least influenced by CYP2C19 metabolism. If PPI therapy with a once daily dosing regimen fails after 5 days of administration, a twice daily dosing regimen with 50% of the initial dose is recommended (e.g. 20 mg twice daily, instead of 40 mg once daily) [12, 14, 15, 27, 47, 48]. If the twice daily dosing regimen shows no or insufficient response, a doubling of the initial dose daily is warranted (e.g. 40 mg twice daily). If this regimen fails after 5 days administration, genotyping for CYP2C19 and consulting a pharmacist (regarding gene-dose effect, co-medication and compliance) is advised. This proposal is meant to be tested in prospective studies to prove that it leads to improved clinical outcomes with better response in patients with acid-related diseases.

Figure 1 Proposal for stepwise individualisation of PPI therapy in Caucasians [12, 14, 15, 27, 47, 48]
REFERENCES

Summary
Proton pump inhibitors (PPIs) are the cornerstone in the treatment of acid-related diseases. PPIs inhibit the secretion of acid, followed by elevation of the intragastric pH. PPIs are generally prescribed in a once daily fixed dose regimen, implying a ‘one dose fits all’ strategy. Although all PPIs are effective acid-suppressive drugs, studies have shown a large inter- and intra-individual variability in response to PPIs. This variability in response to PPIs may lead to an unpredictable effect of the therapy. Three pharmacological parameters may attribute to the variability in response to PPIs: pharmacogenetics, pharmacokinetics and pharmacodynamics. These parameters are described in more detail in chapter 1.

With regard to the pharmacogenetics, CYP2C19 and CYP3A4 are the main enzymes responsible for the metabolism of PPIs. Of these two, genetic variation of CYP2C19 is associated with variation of the clinical effects of PPIs. For CYP3A4 no relevant genetic variations that affect PPI metabolism are known. Subjects with *1/*1 (wildtype/wildtype) genotype for CYP2C19 are considered as homozygous extensive metabolizers (homEMs) associated with normal pharmacokinetics. Their prevalence is 39% in Caucasians. The *2 and *3 variants are held responsible for a decreased metabolism of PPIs resulting in heterozygous extensive metabolizers (hetEMs, *1/*2 or *1/*3 variants) and in poor metabolizers (PMs, *2/*2, *3/*3 or *2/*3 variants). In the Caucasian population about 25% has *1/*2 genotype and 3% has *2/*2 genotype. In contrast to *2 and *3 variants, the *17 variant is associated an increased metabolic rate ((ultra)rapid metabolizers ((U)RM)) and may lead to under treatment in subjects carrying one or two alleles with this variant. About 27% of the Caucasian population has *1/*17 or *17/*17 genotype.

This thesis investigated the role of pharmacogenetics on pharmacokinetics and on pharmacodynamics for better understanding and improvement of therapy with PPIs. The aims of this thesis were:

- to study the occurrence of Rebound Acid HyperSecretion,
- to investigate the speed of onset, the duration of effect and the difference in acid-inhibitory effects between the PPIs esomeprazole, pantoprazole and rabeprazole,
- to study the prevalence of CYP2C19 variants in a Dutch Caucasian population
- to investigate the influence of CYP2C19 polymorphism on the pharmacokinetics and dynamics of PPIs in Caucasian subjects,
- to systematically review the literature about CYP2C19 and PPIs, and
- to develop a fast HPLC analysis for the determination of rabeprazole and its metabolite.

In chapter 2, literature about Rebound Acid HyperSecretion after cessation of PPI therapy is systematically reviewed. Eight studies were included. These studies were heterogenic in design, methods and outcome. There is some evidence from uncontrolled trials for an increased capacity to secrete acid in *H. pylori*-negative subjects after 8 weeks of treatment. Hence, it could be concluded that there is no strong evidence for a clinically relevant increased acid production after withdrawal of proton pump inhibitor therapy.
Variants of CYP2C19 may result in a more rapid or slow metabolism of CYP2C19 substrates. CYP2C19*2 to *6 variant alleles are associated with poor metabolism, whereas CYP2C19*17 alleles are associated with (ultra) rapid metabolism. In chapter 3, we investigated the prevalence of CYP2C19*2 to *6 and *17 variant alleles in a Dutch population. For this purpose, a total of 203 healthy Dutch subjects were genotyped for CYP2C19*2 to *6 and *17 alleles. The DNA samples were genotyped using PCR-RFLP methods. The results showed that the CYP2C19*2 allele frequency was 18%. No *3, *4, *5 and *6 alleles were detected. The allele frequency of CYP2C19*17 was 18%. The frequencies of *1/*1, *1/*2, *2/*2, *1/*17, *2/*17 and *17/*17 genotypes were 39%, 25%, 1.5%, 25%, 7.9% and 1.4%, respectively. It could be concluded that in our Dutch population, no *3, *4, *5 or *6 alleles were observed, indicating an allele frequency < 0.3%. The high frequency of the *17 allele indicates that this allele may be useful as a prognostic factor in predicting the outcome of drugs metabolized by the CYP2C19 enzyme. Our findings are in line with data from Greece and Germany.

In chapter 4 the impact of CYP2C19 variants *2 to *6 and *17 on acid-inhibition and pharmacokinetics of lansoprazole (15 mg, L15), omeprazole (10 mg (O10), 20 mg (O20)) and pantoprazole (40 mg (P40)) in Caucasians was investigated. CYP2C19 genotyping for *2 to *6 and *17 variants was assessed in subjects who were H. pylori negative in two randomized cross-over trials. The influence of CYP2C19 mutations on single and repeated administration of L15 and O10 (study A) and O20 and P40 (study B) was investigated. Pharmacokinetics and the cumulative percentage of time with intragastric pH above 4 (% > pH 4) were assessed on day 1 and 6. In this study, the new parameter ‘Δ percentage of time with intragastric pH > 4’ was introduced. To determine the net response to the study drug, the cumulative percentage of time with pH above threshold 4 at baseline was subtracted from the cumulative percentage of time with pH above threshold 4 at day 1 and day 6 for each individual subject. This gain is represented as Δ percentage of time with intragastric pH > 4. We defined individuals showing a Δ of ≥ 10% as responders and individuals with a Δ of < 10% as nonresponders. The results showed that for study A, CYP2C19 genotyping found five *1/*1, four *1/*2, one *1/*17 and one *2/*17. For study B the results were six *1/*1, two *1/*2, six *1/*17, one *2/*2 and one *2/*17. For all PPIs, AUC was highest in *2/*2 and lowest in *1/*17. On day 1, all PPIs significantly increased % > pH 4 compared with baseline. *1/*1 genotype showed no significant acid-inhibition after L15, O10 and O20. *1/*17 genotype showed no significant acid-inhibition after O20 and P40. *1/*2 genotype showed significant acid-inhibition after L15 and O10. On day 6, all four PPIs showed significantly increased acid-inhibition. *1/*1 and *1/*17 showed a significantly increased % > pH 4 after treatment with O20 and P40. However, in *1/*1 subjects % > pH 4 was not significantly increased after L15 and O10. *1/*2 genotype showed a significant acid-inhibitory effect after repeated dosing with L15 and O10. From these data it was concluded that Caucasian subjects with *1/*1 and *1/*17 genotype need stronger acid-suppression therapy, especially during the first days of treatment or with on-demand therapy.
In the study described in chapter 5, the acid-inhibitory effects of once daily esomeprazole 40 mg and pantoprazole 40 mg for five days were compared at 4, 24 and 120 h after start of oral administration in relation to CYP2C19 genotype and pharmacokinetics. In this study CYP2C19*2 to *6 and *17 genotypes were determined in healthy H. pylori-negative Caucasian subjects. Seven *1/*1, seven *1/*2, two *1/*17, two *2/*17 and one *2/*2 were included in a randomized investigator-blinded cross-over study with esomeprazole 40 mg and pantoprazole 40 mg once daily for 5 days. Intragastric 24-h pH-monitoring was performed on days 0, 1 and 5 of oral dosing. A total of 19 subjects (mean age 24 years, 7 male) completed the study. At day 1 and 5, acid-inhibition with esomeprazole was significantly greater and faster than with pantoprazole. At day 1, 18 out of 19 subjects (95%) showed a response of ≥ 10% with esomeprazole and 14 out of 19 subjects (74%). At day 5, all subjects in the esomeprazole group (100%) and 18 out of 19 subjects (95%) in the pantoprazole group showed a response of ≥ 10%. Differences in acid-inhibition and pharmacokinetics between *1/*1 and *1/*2 genotype were significant for pantoprazole at day 1 and 5. This study showed that esomeprazole 40 mg orally provides acid-inhibition faster than and superior to pantoprazole 40 mg orally after single and repeated administration. The acid-inhibitory effect and the kinetics of pantoprazole are influenced by CYP2C19 genotype.

The acid-inhibitory effects of esomeprazole 40 mg and rabeprazole 20 mg at 4, 24, and 120 hours after oral administration in relation to CYP2C19 genotype are described in chapter 6. CYP2C19*2 to *6 and *17 genotypes were determined in healthy H. pylori-negative Caucasian subjects. Eighteen subjects (mean age 21y, 7 male) with different genotypes (seven *1/*1, seven *1/*2, two *1/*17 and two *2/*17) were included in a randomized investigator-blinded cross-over study with esomeprazole 40 mg and rabeprazole 20 mg. Intragastric 24-h pH-monitoring was performed on days 0, 1 and 5 of oral dosing. The results showed that the onset of acid-inhibition during the first 4 hours after administration did not differ significantly between esomeprazole and rabeprazole. During the upright period, percentage of time with pH > 4 was significantly increased with esomeprazole compared to rabeprazole. At day 1 and 5, acid-inhibition with esomeprazole was significantly greater than with rabeprazole. With esomeprazole, 16 out of 18 subjects (89%) and with rabeprazole, 14 out of 18 subjects (78%) showed a response of ≥ 10% at day 1. At day 5, all subjects in the esomeprazole group (100%) and 17 out of 18 subjects (94%) in the rabeprazole group showed a response of ≥ 10%. Differences in acid-inhibition between *1/*1 and *1/*2 genotype were significant for both PPIs.
The development of a high-speed, high performance liquid chromatography (HPLC) method for the determination of concentrations of rabeprazole and its metabolite rabeprazole thio-ether in the serum of Caucasian individuals is addressed in chapter 7. This fast technique was used because of the unstable properties of rabeprazole and because of the long run times from a previous assay. For the development of this HPLC method, serum concentrations of rabeprazole and rabeprazole thio-ether were determined by liquid-liquid extraction and HPLC with a rapid resolution column. Accuracy and precision of intra-day and inter-day variation, linearity, the lower limit of quantitation (LLOQ), recovery and sample stability were determined as validation parameters. The LLOQ for rabeprazole was 0.015 mg/L (n = 6, CV 11.9%) and 0.026 mg/L for rabeprazole thio-ether (n = 6, CV 12.6%) in human serum. Calibration curves were established between 0.015-1.4 mg/L for rabeprazole and 0.026-0.5 mg/L for rabeprazole thio-ether by non-weighted linear regression. The inter-day correlation coefficients of rabeprazole and its thio-ether were 0.999 or greater. The precision showed a CV of < 0.43%, the bias of intra-day variation was < 11.6% and the bias of inter-day variation was < 12.6%, each tested with n = 6. The accuracy in calf serum showed a CV of < 7.2%. In human serum samples the accuracy was 100.9% for rabeprazole and 98.1% for rabeprazole thio-ether, each tested with n = 6. Frozen quality control samples were stable for at least six months (deviation < 5%). In conclusion: quantitation of rabeprazole and rabeprazole thio-ether by high-speed HPLC method is very fast (a run time < 1.5 minutes), accurate and precise. The method is appropriate for a rapid determination of serum concentrations, especially when there is a large number of samples requiring analysis.

In chapter 8 evidence about the influence of CYP2C19 polymorphism on PPIs is systematically reviewed. Pubmed, Embase and Central were searched up to December 2009 for the indexed terms: “CYP2C19”, “proton pump inhibitors” or “esomeprazole / omeprazole / lansoprazole / pantoprazole / rabeprazole”. Studies were scored with a level of evidence and magnitude. Fourteen studies investigating esomeprazole 40 mg, lansoprazole 30 mg, omeprazole 10 and 20 mg, and rabeprazole 10, 20 and 40 mg were included. In ten studies Japanese subjects were investigated, in two studies Chinese and in two studies Caucasians were involved. The studies focused on intragastric pH and on the proportion of time or percentage during 24 hours with intragastric pH above 3.0 or 4.0. There was evidence of significant influence of CYP2C19 genotypes on these endpoints for lansoprazole, omeprazole and rabeprazole between Asian homEMs and PMs, and between Asian hetEMs and PMs and for pantoprazole between Caucasian homEMs and hetEMs. It was concluded that acid suppression by all PPIs is more or less influenced by CYP2C19 polymorphism, especially after repeated administration with higher doses. Based on this systematic review, the order of CYP2C19 influence between homEMs, hetEMs and PMs for the higher PPI doses is: rabeprazole 20 mg > lansoprazole 30 mg > omeprazole 20 mg > pantoprazole 40 mg > esomeprazole 40 mg. For the lower doses, the order is: omeprazole 10 mg > rabeprazole 10 mg. Considering the small prevalence of PMs in the Caucasian population, genotyping before start of PPI therapy is not useful. The rationale to increase the initial doses of PPIs for Caucasian subjects or to switch to a less CYP2C19-dependent PPI needs further research, especially in homEMs and subjects with *17 variants (RMs).
Chapter 9 discusses the impact of the combined findings of the presented studies on clinical effects of PPIs in the Caucasian population. It showed that all PPIs are more or less influenced by CYP2C19 polymorphism, especially after repeated administration with higher doses. Considering the small prevalence of poor metabolizers in the Caucasian population, genotyping before start of PPI therapy is not useful for Caucasians. Based on our findings two rationales are suggested for Caucasian subjects:

1. To increase the initial doses of PPIs, and/or:
2. To switch to a less CYP2C19-dependent PPI.

Both approaches need further research, especially in homozygous extensive metabolizers and in rapid metabolizers. In addition, in Chapter 9 some comments are given with regard to the applied methodology in the different chapters and recommendations for future research are proposed. Finally, an individualized dosing schedule for Caucasians patients is proposed. In the perspective of our findings, the ‘one dose fits all’ strategy for PPIs may in the near future be changed into a ‘stepwise individualized approach’. This may lead to a further optimization of PPI based therapy in acid-related diseases.
Appendices
Samenvatting
voor niet-ingewijden

Inleiding
Dit proefschrift richt zich op de klinische effecten van proton pomp remmers (PPIs). De toepassing van PPIs vormt de hoeksteen van de behandeling van maagzuur-gerelateerde aandoeningen. PPIs remmen de aanmaak van maagzuur, waardoor de maag minder zuur wordt en patiënten minder last hebben van klachten, als brandend maagzuur. PPIs worden over het algemeen éénmaal daags in een vaste dosering voorgeschreven, een zogeheten one dose fits all strategie. In Nederland zijn vijf PPIs op de markt: esomeprazol (Nexium®), lansoprazol (Prezal®), omeprazol (Losec®), pantoprazol (Pantozol®) en rabeprazol (Pariet®). Esomeprazol, omeprazol en pantoprazol worden het meest gebruikt.

Een maat voor zuur is de pH (hoe zuurder, hoe lager de pH). De effectiviteit van PPIs kan continu gemeten worden door met een electrode de pH in de maag te monitoren. Bij voorkeur wordt eerst 24 uur gemeten zonder PPI gebruik en vervolgens 24 uur na PPI-inname, zodat de zuurremming bepaald kan worden. De uitkomsten van een pH-meting in de maag worden uitgedrukt in twee parameters:
1 de pH in de maag
2 het percentage van de tijd gedurende welke de pH in de maag hoger was dan pH 4 over een 24 uren meetperiode (% van de tijd pH > 4)

Hoewel PPIs effectieve maagzuurremmers zijn, laten onderzoeken zien dat er een groot verschil in respons op PPIs bestaat in en tussen personen (intra- en inter-individuele variabiliteit). Dit verschil in respons leidt tot een onvoorspelbaar effect op de therapie. Drie farmacologische parameters kunnen bijdragen aan deze variabiliteit in respons: farmacodynamiek, farmacokinetiek en farmacogenetica.

De farmacodynamiek beschrijft hoe (en hoe goed) iemand reageert op een geneesmiddel. De farmacokinetiek heeft te maken met hoeveel geneesmiddel zich in het lichaam bevindt (de concentratie in het bloed), de omzetting ervan (metabolisering, hierbij ontstaan omzettingsproducten die wel of niet werkzaam kunnen zijn) en de wijze waarop het geneesmiddel het lichaam verlaat. De farmacogenetica1 richt zich op genetische variatie als oorzaak van verschillen in de effecten van geneesmiddelen.

Dieper ingaand op de farmacogenetica zijn CYP2C19 en CYP3A4 de belangrijkste enzymen in de lever die verantwoordelijk zijn voor de metabolisering van PPIs. Van deze enzymen is CYP2C19 in verband gebracht met de variabiliteit in respons op PPIs. Deze wordt veroorzaakt doordat CYP2C19 genetische varianten heeft. Van het enzym CYP3A4 zijn geen relevante genetische varianten bekend.

Genetische varianten kunnen worden uitgedrukt in een genotype: de door overerving doorgegeven eigenschappen van iemand, aantoonbaar in het DNA. Het deel van het DNA met informatie over één specifieke erfelijke eigenschap wordt gen genoemd. Een gen bestaat over het algemeen uit twee delen: allelen. Wildtype (wt of *1) is de benaming voor het meest voorkomende actieve allele. Van de Kaukasische bevolking heeft 39% het genotype *1/*1 (wt/wt) voor CYP2C19. Mensen met dit genotype worden homozygote extensive metabolizers genoemd. Deze hebben een ‘normale’ farmacokinetiek en een ‘normale’ respons op PPIs.

1 Voor de leesbaarheid wordt in de tekst ervan uitgegaan dat het genotype in deze ook het genoemde fenotype veroorzaakt. Er worden fenotypes genoemd daar waar genotypes correcter zou zijn.
In hoofdstuk 1 wordt nader ingegaan op het verschil tussen genotypes en fentoytes.
De varianten met *2 en *3 worden in verband gebracht met een verminderde omzetting van PPIs. De verminderde omzetting heeft meer blootstelling aan de PPI als gevolg, waardoor de respons groter is. Mensen met deze varianten zijn heterozygote extensive metabolizers (*1/*2 of *1/*3 varianten) of poor metabolizers (*2/*2, *3/*3 en *2/*3 varianten). Van de Kaukasische bevolking heeft circa 25% een *1/*2 genotype en maximaal 3% een *2/*2 genotype. Genotypes met *3 varianten komen nauwelijks voor. In tegenstelling tot de *2 en *3 varianten leiden *17 varianten tot verhoogd metabolisme. Mensen met *17 varianten in hun DNA hebben een versnelde afbraak van PPIs. Dit kan onderbehandeling tot gevolg hebben. Ongeveer 27% van de Kaukasische bevolking heeft een *1/*2 genotype. Zij worden ook wel (ultra)rapid metabolizers genoemd.

Het voorkomen van DNA varianten kan per ras verschillend zijn. Zo is van het Japanse ras bekend dat wildtype DNA bij 35% van deze populatie voorkomt. Circa 55% van de Japanners is heterozygoot extensive metabolizer en 20% is een poor metabolizer. In tegenstelling tot het grote aantal rapid metabolizers in de Kaukatische bevolking telt het Japanse ras slechts 3% van deze groep.

Wanneer men de farmacodynamiek en farmacokinetiek van PPIs wil onderzoeken is het dus van belang om informatie over de genotypes van het te onderzoeken ras voorhanden te hebben. Bij de Kaukatische bevolking zijn tot op heden weinig studies verricht naar de invloed van de verschillende CYP2C19 genotypes op de farmacodynamiek en farmacokinetiek van PPIs.

**Vraagstelling, doel en onderdelen**

De vraagstelling van dit proefschrift is: wat is de invloed van farmacogenetica op de farmacokinetiek en farmacodynamiek van proton pomp remmers. Met als doel om met dit verkregen inzicht een gerichtere behandeling van patiënten met maagzuurgerelateerde aandoeningen te verkrijgen.

Onderdelen van dit proefschrift:
- onderzoek naar optreden van rebound zuursecretie na het staken van een behandeling met PPIs
- onderzoek naar de prevalentie (het voorkomen) van CYP2C19-varianten binnen een Nederlandse (Kaukatische) populatie
- onderzoek naar de invloed van CYP2C19-varianten op de farmacokinetiek en farmacodynamiek van PPIs bij Kaukatische gezonde vrijwilligers
- onderzoek naar het verschil in zuurremmend effect (aanvang, mate en duur van werking) van de PPIs esomeprazol, pantoprazol en rabeprazol in gezonde vrijwilligers
- opzet van een snelle analysemethode voor de bepaling van rabeprazol en metaboliet door gebruik te maken van een zeer snelle high performance liquid chromatography (HPLC) methode
- systematisch literatuuronderzoek over CYP2C19 en PPIs

In **hoofdstuk 2** is systematisch de literatuur over rebound zuursecretie na het stoppen van een PPI behandeling bestudeerd. Deze wordt omschreven als een hogere zuuraanmaak na staken van PPIs in vergelijking met de zuuraanmaak voor de start van behandeling. Er werden acht relevante studies gevonden. Deze waren heterogeen van opzet, methoden en uitkomst. In ongecontroleerde studies bleek er enig bewijs te zijn voor een verhoogde capaciteit om zuur aan te maken na 8 weken behandeling met een PPI. Er was onvoldoende bewijs is voor een klinisch relevante verhoogde zuurproductie na het stoppen van therapie met proton pomp remmers.
In hoofdstuk 3 is de prevalentie onderzocht van CYP2C19 *2 tot *6 en *17-varianten binnen een Nederlandse populatie. Hiervoor is in het DNA van 203 personen het genotype bepaald van de CYP2C19 *2 tot *6 en *17-varianten. De resultaten laten een frequentie van het *2 allele zien van 18%. De allelfrequentie van de *17 variant was 18%. Er zijn geen *3, *4, *5 en *6 allelen gevonden. De prevalentie van *1/*1, *1/*2, *2/*2, *1/*17, *2/*17 en *17/*17 genotypes was respectievelijk 39%, 25%, 1.5%, 25%, 7.9% en 1.4%. De hoge frequentie van het *17-allele laat zien dat dit allele van waarde kan zijn bij het voorspellen van het effect van geneesmiddelen die via CYP2C19 worden gemetaboliseerd. Deze bevindingen zijn in overeenstemming met onderzoeksresultaten uit Griekenland en Duitsland.

In hoofdstuk 4 is de invloed van de CYP2C19-varianten *2 tot *6 en *17 op de zuurremming en farmacokinetiek van oraal lansoprazol 15 mg, omeprazol 10 mg en 20 mg en pantoprazol 40 mg onderzocht. Het CYP2C19 genotype is bepaald bij proefpersonen die deelgenomen hebben aan twee eerder uitgevoerde studies. De invloed van het CYP2C19 genotype op lansoprazol 15 mg en omeprazol 10 mg (studie A) en omeprazol 20 mg en pantoprazol 40 mg (studie B) is onderzocht na éénmalige en na herhaalde toediening. De farmacokinetiek en het percentage van de tijd met pH > 4 in de maag zijn bestudeerd op dag 1 en dag 6 van inname. In dit hoofdstuk is een nieuwe parameter geïntroduceerd. Om de respons op de PPIs te bepalen is van iedere proefpersoon het cumulatieve percentage van de tijd met pH > 4 tijdens de baseline meting (zonder PPI) afgetrokken van het cumulatieve percentage van de tijd met pH > 4 op dag 1 en dag 6 (met PPI). De uitkomst is weergegeven als Δ (delta) percentage van de tijd met pH > 4. Personen met een Δ van ≥ 10% zijn vervolgens gedefinieerd als responders en personen met een Δ < 10% als non-responders.

Bij de deelnemers aan studie A waren vijf homozygote extensive metabolizers, vier heterozygote extensive metabolizers en twee (ultra)rapid metabolizers. Aan studie B namen 6 homozygote extensive metabolizers, twee heterozygote extensive metabolizers, zeven rapid metabolizers en één poor metabolizer deel. Bij alle PPIs was de blootstelling aan het geneesmiddel het grootst bij de poor metabolizers en het kleinst bij de rapid metabolizers. Op dag 1 van de toediening lieten alle PPIs een significant verhoogd percentage van de tijd met pH > 4 zien. Uitgesplitst naar genotype trad geen significante zuurremming van de PPIs op bij homozygote extensive metabolizers na inname van lansoprazol 15 mg, omeprazol 10 mg of omeprazol 20 mg. Rapid metabolizers lieten geen significante zuurremming zien na omeprazol 20 mg en pantoprazol 40 mg. Heterozygote extensive metabolizers toonden wel significante zuurremming na lansoprazol 15 mg en omeprazol 10 mg. Op dag 6 lieten alle PPIs een significant verhoogd percentage van de tijd met pH > 4 zien. Dit bleek echter, uitgesplitst naar genotype, niet significant voor homozygote extensive metabolizers die lansoprazol 15 mg en omeprazol 10 mg hadden ingenomen.

Deze bevindingen leidden tot de conclusie dat Kaukasische rapid en homozygote extensive metabolizers sterkere zuurremming nodig hebben, zeker gedurende de eerste dagen van de therapie of bij on-demand (alleen inname van PPI bij klachten, op eigen initiatief van de patiënt) therapie.

Hoofdstuk 5 beschrijft de studie waarin de zuurremmende effecten van oraal esomeprazol 40 mg en pantoprazol 40 mg zijn vergeleken 4, 24 en 120 uur na toediening, in relatie tot het CYP2C19 genotype en de farmacokinetiek. Ook in deze studie zijn de CYP2C19 genotypes bepaald bij gezonde Kaukasische vrijwilligers. Zeven homozygote extensive metabolizers, zeven heterozygote extensive metabolizers, vier rapid metabolizers en één poor metabolizer maakten deel uit van een studie naar esomeprazol 40 mg en pantoprazol 40 mg eenmaal daags, gedurende vijf dagen. Meting van de pH in de maag vond plaats gedurende 24 uur op dag 0, dag 1 en dag 5 van toediening.
In totaal hebben 19 proefpersonen tot het eind aan de studie deelgenomen. Zowel na 4 uur, 24 uur en 120 uur was de zuurremming met esomeprazol significant beter en sneller dan met pantoprazol. Op dag 1 liet 95% van de proefpersonen een respons zien na esomeprazol, in vergelijking met 74% na pantoprazol. Op dag 5 liet het percentage op tot 100% na esomeprazol en 95% na pantoprazol. Pantoprazol liet significante verschillen zien in zowel zuurremming als farmacokinetiek tussen de homozygote en heterozygote extensieve metabolizers op dag 1 en dag 5. Hierbij lieten de heterozygote extensieve metabolizers een grotere blootstelling en betere zuurremming zien dan de homozygote extensieve metabolizers. Bij esomeprazol is geen verschil gemeten tussen de homozygote en heterozygote extensieve metabolizers.

Samenvattend, de studie toonde aan dat esomeprazol 40 mg snellere en superieure zuurremming biedt vergeleken met pantoprazol 40 na zowel éénmalige als herhaalde toediening. Bij pantoprazol worden, in tegenstelling tot bij esomeprazol, zowel de zuurremmende effecten als de kinetiek beïnvloed door CYP2C19 genotype.

In hoofdstuk 6 wordt een studie beschreven naar de zuurremmende effecten van esomeprazol en rabeprazol in verschillende genotypen. Voor deze studie zijn eveneens de CYP2C19 genotypes bepaald bij gezonde vrijwilligers. Zeven homozygote extensieve metabolizers, zeven heterozygote extensieve metabolizers en vier rapid metabolizers maakten deel uit van deze studie naar esomeprazol 40 mg en rabeprazol 20 mg eenmaal daags oraal, gedurende vijf dagen. Meting van de pH in de maag vond plaats gedurende 24 uur op dag 0, dag 1 en dag 5 van toediening. In totaal hebben 18 proefpersonen het volledige studie protocol doorlopen.

De resultaten laten zien dat de snelheid van werking gedurende de eerste 4 uur na inname niet significant verschilde tussen esomeprazol en rabeprazol. Op dag 1 en dag 5 was zuurremming met esomeprazol significant beter dan met rabeprazol. Op dag 1 werd een respons bereikt bij 89% in de esomeprazolgroep en bij 78% in de rabeprazol-groep. Op dag 5 werd deze respons bereikt bij 100% in de esomeprazolgroep en bij 94% in de rabeprazolgroep. Zowel esomeprazol als rabeprazol lieten bij heterozygote extensieve metabolizers een betere zuurremming zien dan de homozygote extensieve metabolizers.

Deze studie heeft aangetoond dat esomeprazol 40 mg superieure zuurremming biedt vergeleken met rabeprazol 20, zowel na éénmalige als herhaalde toediening. De zuurremmende effecten van zowel esomeprazol als rabeprazol worden beïnvloed door CYP2C19 genotype.

In hoofdstuk 7 wordt een analysemethode beschreven om concentraties rabeprazol en de metaboliet (rabeprazol thio-ether; in deze metaboliet wordt rabeprazol omgezet nadat het in het bloed is opgenomen) in bloedmonsters van proefpersonen te kunnen bepalen. Hierbij is gebruik gemaakt van een snelle techniek die high speed HPLC heet. Deze is ontwikkeld omdat rabeprazol niet stabiel is in bloed. Bovendien duurden eerder beschreven analyses erg lang (meer dan 40 minuten per monster).

Om deze high speed methode te kunnen valideren zijn de juistheid en precisie van intra- en inter-dagvariatie, onderste bepalingsgrens, opbrengst en stabilitéit van de monsters onderzocht. De intra- en inter-dagvariatie, opbrengst en stabilitéit voldeden aan de eisen. De onderste bepalingsgrens was 0,015 mg/L voor rabeprazol en 0,026 mg/L voor rabeprazol thio-ether.
Concluderend kan worden gesteld dat de bepaling van rabeprazol en rabeprazol thioether met high-speed HPLC snel (< 1,5 minuten), accuraat en precies is. De methode is aan te bevelen wanneer grote hoeveelheden monsters onderzocht moeten worden.

**Hoofdstuk 8** geeft de resultaten weer van een literatuuronderzoek naar de invloed van CYP2C19 polymorfisme op PPIs. Hiervoor zijn databases als Pubmed, Embase en Central tot december 2009 doorzocht op de geïndexeerde termen ‘CYP2C19’, ‘proton pomp remmers, of esomeprazol / omeprazol / lansoprazol / pantoprazol / rabeprazol’. Veertien studies met PPIs zijn gescroond op basis van kwaliteit en relevantie van de uitkomsten. Dit waren studies over esomeprazol 40 mg, lansoprazol 30 mg, omeprazol 10 en 20 mg en rabeprazol 10, 20 en 40 mg. Tien studies waren uitgevoerd bij Japanners, twee bij Chinezen en twee bij Kaukasiërs. De studies gebruikten als uitkomstmatten de pH in de maag en het aantal uur (of het percentage van de tijd) dat de pH in de maag groter was dan 3,0 of 4,0. In deze studies werd gevonden dat alle PPIs in meer of mindere mate beïnvloed worden door CYP2C19 varianten, met name na herhaalde dosering en bij hogere doseringen.

Op basis van de uitkomsten van dit literatuuronderzoek kan gesteld worden dat de volgorde van invloed van CYP2C19 op PPI 20 mg > lansoprazol 30 mg > omeprazol 20 mg > pantoprazol 40 mg > esomeprazol 40 mg. Voor de lagere doseringen geldt dat omeprazol 10 mg meer beïnvloed wordt dan rabeprazol 10 mg.

Gezien de lage prevalentie van poor metabolizers in de Kaukasische bevolking is standaard het genotype bepalen vóór aanvang van een PPI behandeling niet zinvol. Verder is vooral voor rapid metabolizers en homozygote extensive metabolizers nader onderzoek geïndiceerd naar het verhogen van de initiële doseringen van PPIs en het switchen naar de PPI die het minst gevoelig is voor CYP2C19.

**Discussie en conclusies**

In **hoofdstuk 9** worden de bevindingen van de eerdere hoofdstukken over de klinische effecten van PPIs bij de Kaukasische bevolking samengevat. De resultaten van dit proefschrift laten zien dat alle PPIs in meer of mindere mate beïnvloed worden door CYP2C19 varianten, in het bijzonder na herhaalde toediening met hogere doses. Wanneer de lage prevalentie van poor metabolizers in de Kaukasische bevolking in aanmerking genomen wordt, is het standaard bepalen van het genotype voor aanvang van PPI therapie niet zinvol binnen deze bevolkingsgroep.

Op grond van bovenstaande bevindingen worden twee strategieën voorgesteld:

1) verhogen van de initiële dosering van PPIs en/of:
2) switchen naar de PPI die het minst gevoelig is voor CYP2C19.

Beide benaderingen dienen nader te worden onderzocht, vooral bij homozygote extensive metabolizers en (ultra)rapid metabolizers.

Er wordt verder commentaar gegeven op de toegepaste methodologie in de verschillende hoofdstukken en er worden aanbevelingen gedaan voor toekomstig onderzoek. Het hoofdstuk eindigt met een voorstel voor een geïndividualiseerd doseerschema voor Kaukasische patiënten. In het licht van onze bevindingen zou de one dose fits all strategie in de nabije toekomst dienen te veranderen in een stapsgewijze individuele dosering.

Deze verandering zal leiden tot verdere optimalisatie van behandeling met proton pomp remmers bij patiënten met maagzuurgereleateerde aandoeningen.
Dit proefschrift is tot stand gekomen door samenwerking tussen en met vele mensen. Vanzelfsprekend dient het meest gelezen onderdeel van een dissertatie een groot doel: iedereen bedanken- in willekeurige volgorde- die een bijdrage heeft geleverd aan mijn boekje!

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Den Haag, juli 2010

Nicole
List of publications related to this thesis


HagaZiekenhuis van Den Haag


STZ-ziekenhuis: teaching hospital
Het HagaZiekenhuis verleent als STZ-ziekenhuis (Samenwerkende Topklinische opleidingsZiekenhuizen) hooggespecialiseerde medische zorg. Als “Teaching Hospital” voelt het HagaZiekenhuis zich verantwoordelijk voor onderwijs en opleidingen in brede zin, het bevorderen van hoogwaardige patiëntenzorg, topklinische behandeling en topreferente zorg en toegepast wetenschappelijk onderzoek en zorginnovatie.

Opleiding, onderwijs en onderzoek

Het HagaZiekenhuis vervult, in samenwerking met de universitaire medische centra, een belangrijke rol in toegepast medisch wetenschappelijk onderzoek. Gezien de aard van de patiëntenpopulaties is het HagaZiekenhuis bij uitstek geschikt voor participatie in grootschalig multicenter onderzoek en medical technology assessment (MTA) en is er gelegenheid voor promotieonderzoek van arts-assistenten en specialisten. Voor meer informatie: www.hagaziekenhuis.nl