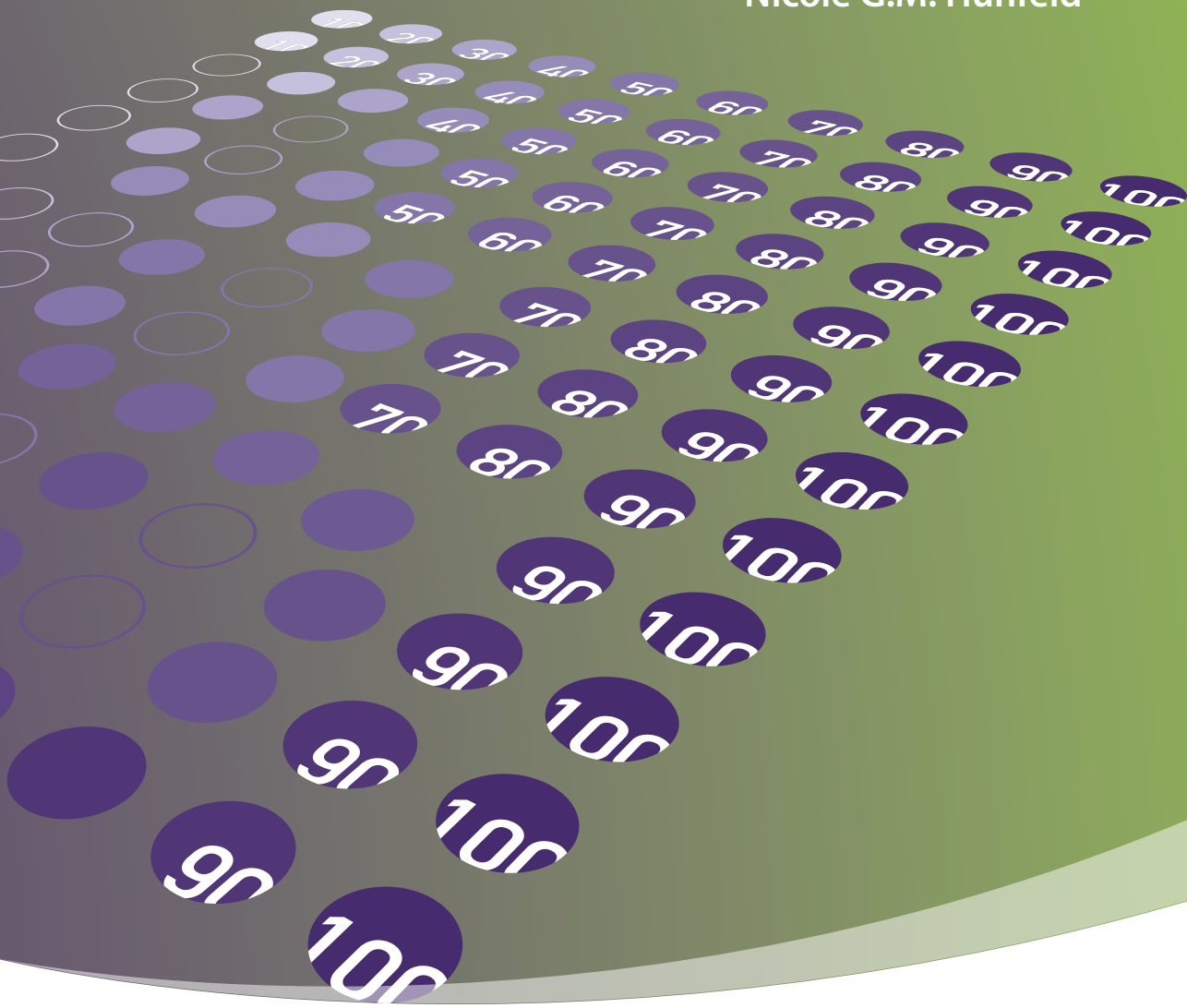
Clinical effects of proton pump inhibitors

Focus on pharmacogenetics, kinetics and dynamics

Nicole G.M. Hunfeld



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Het HagaZiekenhuis van Den Haag is trots op medewerkers die fundamentele bijdragen leveren aan de wetenschap en stimuleert hen daartoe. Om die reden biedt het HagaZiekenhuis zijn promovendi de mogelijkheid hun dissertatie te publiceren in een speciale Haga uitgave, die onderdeel is van de promotiereeks van het HagaZiekenhuis. Daarnaast kunnen promovendi van het HagaZiekenhuis in het wetenschaps-magazine HagaScoop van het ziekenhuis aan het woord komen over hun promotieonderzoek.

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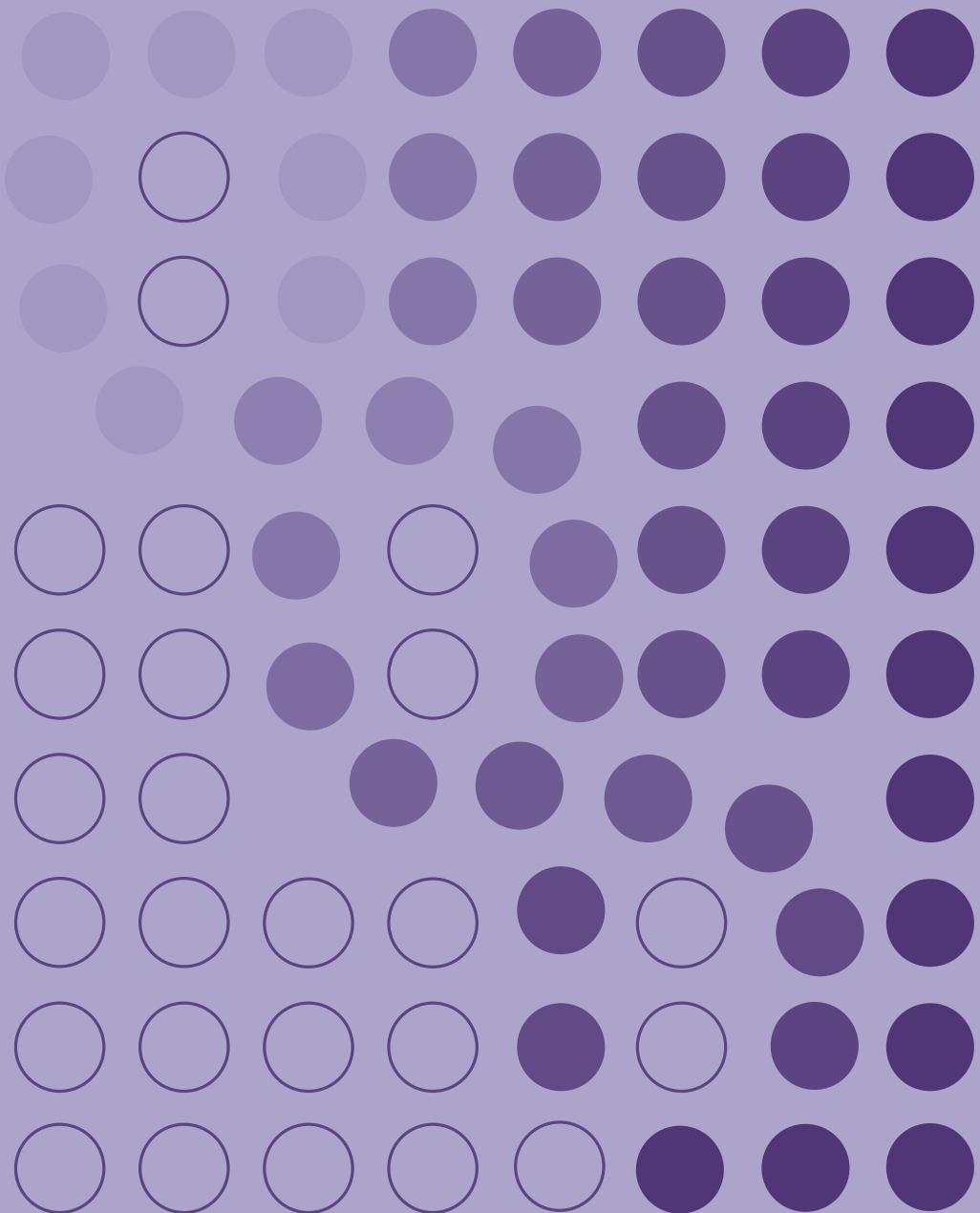

General introduction

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1 General introduction

Acid-related diseases

Acid-related diseases, including gastroesophageal reflux disease (GERD), peptic ulcer disease (PUD), and dyspepsia are common. Of every 1000 patients visiting a general practitioner in the Netherlands, 34 do so because of upper gastro intestinal symptoms [1]. These symptoms include heartburn, acid regurgitation, abdominal or retrosternal discomfort or pain, bloating, nausea, globus feeling, and dysphagia. It is estimated that 20-30% of these symptoms are caused by GERD, about 5% by peptic ulcer disease and less than 1% by a malignancy [2]. In 60 to 70% of the patients, no pathophysiological cause is identified and these patients are often classified as having functional upper GI symptoms. Because of their persistent or recurrent natural history, the acid-related disorders are associated both with diminished quality of life and significant morbidity [3].

Gastro-oesophageal reflux disease

Gastro-oesophageal reflux disease is a condition characterized by pathologic reflux from the stomach into the oesophagus leading to symptoms and/or esophageal lesions. It comprises a wide spectrum of disorders, ranging from gastro-oesophageal reflux without significant clinical or pathological impact, through to the more severe complications of reflux disease, including erosive oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma [4]. Symptoms considered to be related to reflux of gastric contents into the oesophagus are common in the general population. The typical symptoms of GERD in adult patients are retrosternal or sub-sternal burning, regurgitation, epi-gastric pain and dysphagia. The atypical symptoms include belching, water brash, wheezing and cough. These symptoms appear to be more commonly experienced in developed countries, although their incidence and prevalence now appear to be increasing in parts of the world where they were previously uncommon, particularly South-East Asia and the Far East. In Western populations it is estimated that one in four persons experiences heartburn or acid regurgitation at least once a month, 12% at least once per week and 5% on a daily basis [5].

Peptic ulcer disease

A peptic ulcer is a defect in the gastric or duodenal mucosa that extends into the muscularis mucosae. Peptic ulcers occur mainly in the stomach (gastric ulcer; GU) or proximal duodenum (duodenal ulcer; DU). PUD develops when the protective mechanisms of the gastrointestinal mucosa, such as mucus and bicarbonate secretion, are overwhelmed by the damaging effects of gastric acid and pepsin. Two decades ago, *Helicobacter pylori (H. pylori)* infection was identified as the main cause of PUD. Management of *H. pylori*-associated PUD has improved radically since then. As the prevalence of *H. pylori* infection has declined in Western countries, GU has become more commonly associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (ASA). In the Netherlands, the incidence of gastric ulcers decreased between 1992 and 2003 from 18.3 to 6.8/100,000 in men and from 13.0 to 5.1/100,000 in women [6]. However, prescriptions for drugs implicated in the aetiology of PUD, such as aspirin and NSAIDs, have also increased over this time period [7]. Individuals with PUD are at risk of developing complications such as gastroduodenal haemorrhage, perforation and obstruction. Mortality among patients with these complications is high. According to Dutch guidelines, patients with risk-factors (age > 70 years, PUD, ongoing *H. pylori* infection, use of anticoagulants, severe rheumatoid arthritis, heart failure, diabetes, high dose NSAID, use of corticosteroids and/or SSRI's) using NSAIDs or ASA should be prescribed a gastroprotective drug [8]. Still, adherence to gastroprotection for prevention of NSAID-induced PUD remains far from optimal [9].

Dyspepsia

Dyspepsia is a chronic or recurrent pain or discomfort centered in the upper abdomen. Discomfort is defined as a subjective negative feeling that is nonpainful, and can incorporate a variety of symptoms including early satiety or upper abdominal fullness. Frequent reflux symptoms (twice a week or more) probably impair quality of life and are generally considered to identify GERD until proven otherwise [10]. In the Netherlands, 150 new dyspeptic patients present at general practices annually [11].

TREATMENT OF ACID-RELATED DISEASES

Antacids

Antacids are alkali preparations that neutralize hydrochloric acid in the stomach. Antacids can contain aluminium, magnesium, calcium or combined substances. Antacids are indicated for dyspepsia, GERD, reflux oesophagitis and gastritis. Their onset of action is fast, but they require frequent administration (4 to 6 times a day) because of their short duration of action [12, 13].

H_2-receptor antagonists

Parietal cells in the stomach express receptors for acetylcholine, gastrin and histamine. Stimulation of these receptors results in gastric acid production. H_2-receptor antagonists (H_2RAs) inhibit acid production by reversibly competing with histamine for binding to H_2-receptors on the parietal cells. Four different H_2RAs are available: cimetidine, famotidine, nizatidine and ranitidine. H_2RAs are indicated for reflux-oesophagitis, ulcus duodeni, ulcus ventriculi, prevention of recurrent peptic ulcers and the treatment of NSAID related ulcers. These agents are primarily effective in decreasing basal acid production and nocturnal acid breakthrough. They are however less effective in controlling food-stimulated acid secretion during daytime. In general, H_2RAs are administered twice a day. Although H_2RAs have reasonable efficacy, patients develop tolerance in particular with continuous therapy [12-15].

Proton pump inhibitors

Proton pump inhibitors (PPIs) suppress gastric acid secretion by specific inhibition of the H^+/K^+- ATPase in the gastric parietal cell. This process starts with absorption of the PPI in the parietal cell. PPIs are weak bases, so protonation takes place in the acidic region of the secretory canaliculus of the parietal cell. In the secretory canaliculus, the methylsulfinylgroup shifts to a highly reactive sulfenamide. The final step is covalent binding of the reactive sulfenamide to 2 cysteine moieties of the catalytic subunit of the H^+/K^+-ATPase of the proton pump. This results in inhibition of the acid secretion, followed by elevation of the intragastric pH [16].
PPIs are indicated for the treatment of GERD, reflux oesophagitis, peptic ulcers and Zollinger-Ellison syndrome. In addition, PPIs are used for gastroprotection in patients using NSAIDs or ASA. In combination with two suitable antibiotics, PPIs are also used for the eradication of *H. pylori* infection. In the Netherlands five PPIs are available: esomeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole. Their registered indications for oral administration are shown in Table 1.

Table 1 PPIs and their indications for oral administration

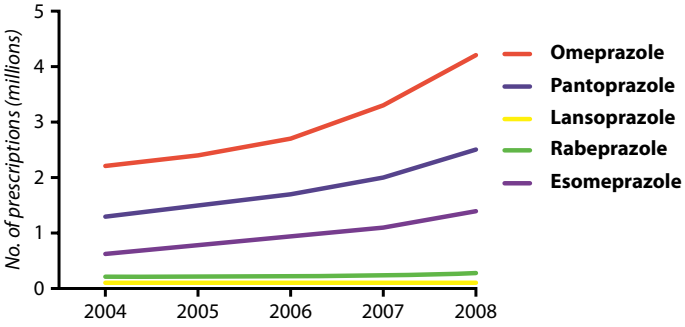
	Esomeprazole Nexium®	Lansoprazole Pariet®	Omeprazole Losec MUPS®	Pantoprazole Pantozol®	Pantoprazole Pantozol®	Rabeprazole Pariet®
Dose *(mg)*	20,40	15,30	10,20,40	20	40	10,20
Indication						
Duodenal ulcer	X[1]	X	X		X	X
Gastric ulcer		X	X		X	X
H. pylori eradication	X	X			X	X
Reflux oesophagitis initial	X	X	X*		X	
Reflux oesophagitis maintenance	X			X		
Acid-related dyspepsia			X			
GERD initial treatment			X	X		X
GERD maintenance treatment		X				X
GERD symptomatic treatment	X	X		X		
NSAID related ulcers treatment	X					
NSAID gastro-protection	X		X	X		
Zollinger-Ellison syndrome	X	X	X		X	X

** also in children > 1 year,*

[1] H. pylori associated

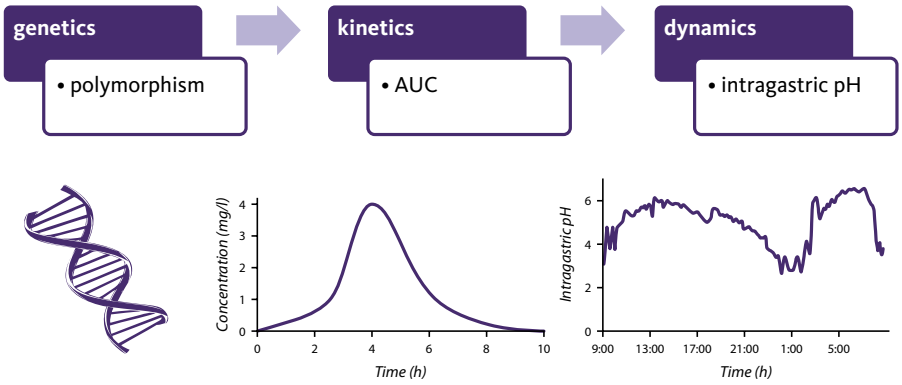
A Dutch cohort study including 16 311 new PPI users showed that the most frequent indications for PPI use were GERD (27%), nonreflux dyspepsia (25%), and *H. pylori*-associated indications (15%). In 21% of patients, PPIs were given for the prevention or treatment of NSAID- or ASA related gastrointestinal complications. About 6% of patients used PPIs for other reasons, whereas for 7% no indication was recorded [9]. The number of PPI prescriptions in the Netherlands from 2004 to 2008 are shown in Figure 1 [17]. To indicate its impact, 7.8 million prescriptions in 2008 involved PPIs and its volume is growing (up to 16% per year) [18].

Figure 1 Number of PPI prescriptions in the Netherlands from 2004 to 2008



In the management of acid-related diseases, PPIs are mostly prescribed for once daily use. Some speculate that all PPIs show similar efficacy on a milligram to milligram basis [19]. With the once daily dosing therapy and in therapy with the same amount of mg, studies however showed a large variability in response to PPIs [20, 21]. This variability may lead to an unpredictable effect of the therapy. In general, three pharmacological parameters may attribute to the response to PPIs: pharmacogenetics, pharmacokinetics and pharmacodynamics (Figure 2). More insight in the role of these parameters is needed for better understanding and improvement of therapy with PPIs.

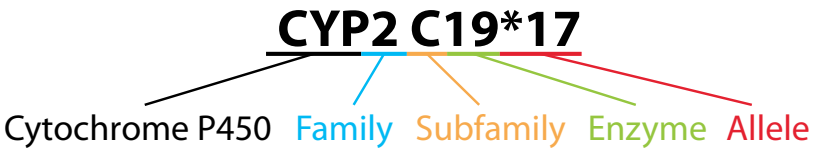
Figure 2 Relationship between pharmacogenetics, pharmacokinetics and pharmacodynamics. (AUC= Area Under the Curve)



PHARMACOGENETICS AND PPIs

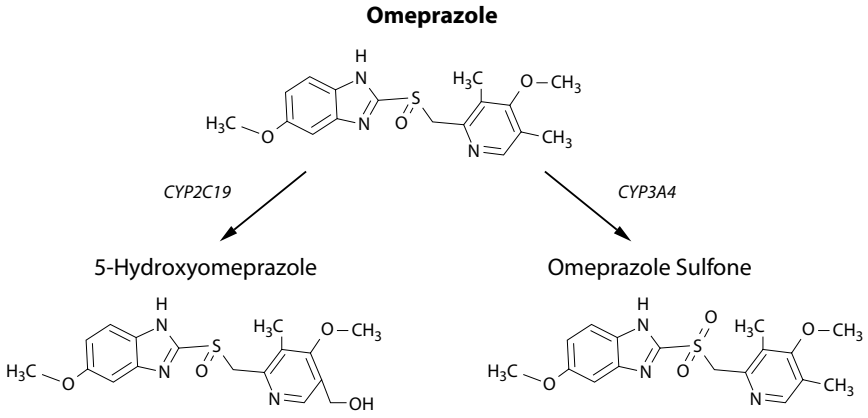
Pharmacogenetics (PG) explains how people respond in different ways to the same drug treatment because of their genetic profile. About 20 years ago, it was discovered that PPIs are susceptible to pharmacogenetic variations [22]. Much of the current clinical research in pharmacology is at the level of PG. It explores variation in genes involved in drug metabolism with a particular emphasis on improving drug safety and characterization of therapeutic failure. From a more pharmacological point of view, PG are the basis for pharmacokinetic variances that determine the amount of drug that is ready for absorption into the blood and its clearance from the blood.
Oral medications are metabolized in the bowel wall and in the liver. This is known as the "first pass" effect. This first pass metabolism is characterized by two phases of enzymatic reactions. Phase I consists of oxidation, hydroxylation, reduction or hydrolysis. During phase I, CYP isoenzymes are responsible for oxidation of many drugs. CYP isoenzymes are a group of heme-containing enzymes embedded primarily in the hepatocytes (liver cells) (Figure 3) [23].

Figure 3: CYP enzymes and their nomenclature



PPIs are metabolized by CYP2C19 and CYP3A4 (Figure 4). CYP2C19 is the main enzyme involved in metabolism of PPIs and shows genetic variation [24]. Subjects with normal (non-mutated) alleles for CYP2C19 are referred to as wildtype/wildtype (*wt/wt* or **1/*1*) genotype. This is associated with a homozygous extensive metabolizer phenotype. Several single nucleotide polymorphic variants (SNPs) of the CYP2C19 gene have been identified that influence the capacity to metabolize PPIs [25]. *CYP2C19*2*, **3*, **4*, **5* and **6* mutations 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 [23, 25, 26]. Studies in Japanese subjects have shown that subjects with **2* or **3* mutations for CYP2C19 respond better to PPIs than subjects without these mutations [25, 27]. When these subjects possess one mutation, their genotype is known as *wt/*2* (or *wt/*3*), accompanied by a heterozygous extensive metabolizer phenotype. With two mutated alleles, their genotype can be **2/*2* (or **2/*3* or **3/*3*). These genotypes are referred to as the poor metabolizer phenotype. In contrast, some mutations lead to a decreased response to PPIs. *CYP2C19*17* mutations are associated with increased metabolism of omeprazole. This may result in lower blood levels (and/or lower AUCs) and reduced acid inhibition [28, 29]. The *wt/*17* or **17/*17* genotype is associated with an (ultra)rapid metabolizer phenotype.

Figure 4 Metabolism of omeprazole by CYP enzymes in the liver



The prevalence of CYP2C19 mutations differs among populations. In Eurasia an increase in **2* and **3* mutations is seen from West to East. In the Caucasian population about 30 to 40% has *wt/*2* genotype and 2 to 5% has **2/*2* genotype [30]. In the Chinese population, about 50% has *wt/*2* or *wt/*3* and 24% has **2/*2*, **2 /*3* or **3 /*3* genotype. The prevalence of *CYP2C19*17* mutation is the opposite. About 36% of the Caucasian population has *wt/*17* or **17/*17* genotype compared to 8% of the Chinese and 1% of the Japanese population [28, 31]. In contrast to the Asian populations, the impact of CYP2C19 on PPIs in Caucasian subjects has not been intensively studied yet.

PHARMACOKINETICS OF PPIs

Pharmacokinetics (PK) describes the processes a drug is subject to in the body. After first pass metabolism in the liver, PPIs enter the systemic circulation and are cleared by hepatic metabolism. This results in a concentration of the PPI in blood that can be measured. This process of absorption and elimination can be described by the pharmacokinetics. The values for the main pharmacokinetic parameters for PPIs are shown in Table 2. The maximal serum drug concentration (C_{max}) among PPIs varies widely depending on the rate of passage in the gastrointestinal tract, release of drug, intraduodenal pH and first pass effect [32]. The oral bioavailabilities (*F*) of the PPIs differ significantly [33]. The *F* of omeprazole and esomeprazole is initially low due to chemical acid degradation. After single dose, *F* is approximately 35% for omeprazole and 64% for esomeprazole. After repeated dosing, *F* increases to 60% and 94% respectively [34-37]. Pantoprazole, lansoprazole and rabeprazole have a constant bioavailability irrespective of single or repeated dosing. The timepoint at which the maximal serum concentration occurs (t_{max}) varies from 0.5 to 3.5 hours. All PPIs have a plasma half-life of elimination ($t_{1/2}$) of approximately 1 hour. All PPIs are highly protein bound (> 95%) and rapidly metabolized in the liver into non-active metabolites. Their renal clearance is negligible.

Table 2 PPIs and their pharmacokinetics after oral administration

	Esomeprazole 40 mg MUPS	Lansoprazole 30 mg capsule	Omeprazole 20 mg MUPS	Pantoprazole 40 mg tablet	Rabeprazole 20 mg tablet
Bioavailability (*F*) (%)	64 (sd) / 94 (md)	80-90 (sd)	35 (sd) / 60 (md)	77	52
t_{max} (h)	1.6	1.5-3	0.5-3.5	2.5	3.5
C_{max} (mg/L)	1.6	0.6-1.2	0.67 (sd) / 1.5 (md)	2-3	0.41
Vd (L/kg)	0.22	0.39	0.3	0.15	-
Cl (L/h)	17 (sd) / 9 (md)	49	30 to 36	7	18
$t_{1/2}$ (h)	1.5	1-2	0.5-1	1	1
AUC (mg*h/L)	1.49 (sd) / 3.87 (md)	3.83	0.65 (sd) / 1.13 (md)	4.34	0.90
References	[38-40]	[33, 39-42]	[39, 40, 43, 44]	[39, 40, 44, 45]	[39, 40, 46]

sd: single dose, md: multiple dose

PHARMACODYNAMICS OF PPIs

While the pharmacokinetics describe what the body does to the drug, the pharmacodynamics (PD) explore what a drug does to the body. PPIs in the systemic circulation are available for binding to the gastric H^+/K^+-ATPase of the proton pumps. This results in inhibition of the acid secretion, followed by elevation of the intragastric pH. pH metry is the most frequently applied method to study the efficacy of acid-inhibitory drugs continuously. pH metry is a technique that measures the pH by a probe placed in the oesophagus or stomach. This technique is shown to be suitable for detection of small changes in pH, especially when the probe is placed in the stomach (10 cm below the lower oesophageal sphincter). pH metry obtains a profile of intragastric pH over a 24-hour time period (Figure 5). Intragastric pH is measured by a miniature glass or antimony electrode connected to a portable datalogger with a sampling rate of 4 per second. Every two seconds, the median of 8 voltage measurements is calculated and stored. Data analysis is based on median pH values over 6 seconds.

To describe the dose-effect relationship of acid-inhibitory drugs 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 is the cumulative percentage of time that intragastric pH value is above or below pH threshold 4 (% time > pH 4 or % time < pH 4) [47]. The acid production of a healthy individual is considered normal when pH is below 4 for more than 70% of time ("baseline measurement"). This parameter is used as an inclusion criterion in clinical studies.
There is a poor correlation between the maximal serum concentration (t_{max}) and the degree of acid suppression in studies with omeprazole. However, the area under the plasma concentration-time curve correlates well with acid suppression with the PPIs omeprazole and esomeprazole [34, 48]. Unfortunately, information about the relationship between the pharmacokinetics and pharmacodynamics of the other PPIs is lacking. Table 3 shows the median intragastric pH and the percentage of time with pH > 4 after oral administration of PPIs in healthy *H. pylori*-negative subjects, based on data from a small selection of publications.

Figure 5 pH-metry and an example of a 24-hour intragastric pH profile in a healthy subject (baseline)

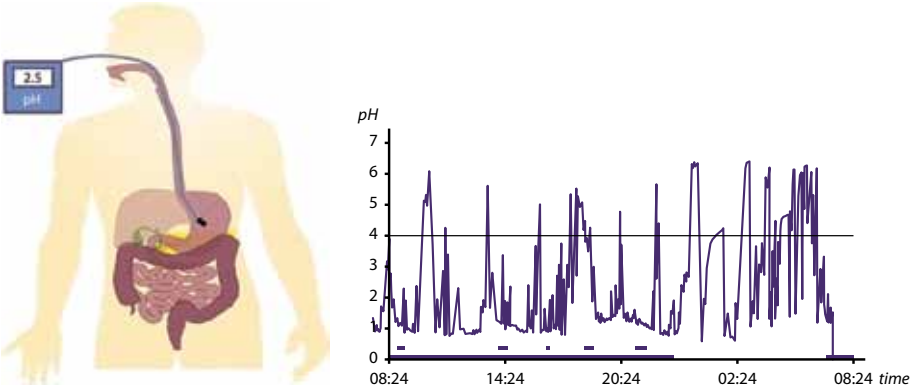


Table 3 Indication of median intragastric pH and percentage of time with pH > 4 of PPIs in healthy *H. pylori*-negative subjects

	Day	Esomeprazole 40 mg MUPS	Lansoprazole 30 mg capsule	Omeprazole 20 mg MUPS	Pantoprazole 40 mg tablet	Rabeprazole 20 mg tablet
Median intragastric pH	1	3.1	3.8	2.0	2.4	3.2
	5-8	4.7 (5)*	3.8 (7)	4.1 (6)	3.7 (6)	4.7(8)
% time pH > 4	1	37	51	21	30	44
	6	64 (5)*	49 (7)	52 (6)	46 (6)	60 (8)
Number of subjects		39	12	16	16	24
References		[49]	[50]	[44]	[44]	[51]

No baseline data are displayed.

** numbers between brackets indicate the days of administration.*

Rebound Acid HyperSecretion

Raising a subjects intragastric pH is the primary goal in acid-related diseases. However, there are speculations that this may lead to an overstimulation of acid production after the drug is stopped, as is shown for H_2-receptor antagonists (especially ranitidine) [52, 53]. This process is called rebound acid hypersecretion (RAHS). If RAHS would occur after cessation of PPIs, it might have consequences for patients because of aggravation of complaints. It has been suggested that the introduction of stronger acting PPIs, like esomeprazole, would more rapidly induce RAHS [54]. In literature, there are few clinical data about the occurrence of RAHS after stopping the intake of PPIs. Its existence and occurrence need further investigation.

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AIMS AND OUTLINE (see Figure 6):

This thesis starts with the description of the role of PPIs in the therapy of acid-related disorders and with an explanation of the terms pharmacogenetics, kinetics, dynamics (including RAHS) (Chapter 1). Several important questions concerning these issues are further addressed in the thesis:

Serious questions have been raised whether cessation of PPI therapy results in RAHS. With the introduction of stronger acting PPIs, like esomeprazole, theses questions needed to be answered. In this perspective, we conducted a systematic review of literature about RAHS after cessation of PPI therapy (Chapter 2).

Variants of CYP2C19 may result in a decreased or increased 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. The presence of these polymorphisms thus impacts the efficacy of drugs which are metabolized by CYP2C19. The prevalence of *CYP2C19*2* to **6* and **17* variant alleles in the Dutch population is studied in Chapter 3.

Most studies investigating the influence of CYP2C19 variants on the pharmacokinetics and dynamics of PPIs were performed in selected groups of non-Caucasian subjects. No information about the influence of CYP2C19 genotype on the dynamics 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 lansoprazole, omeprazole and pantoprazole in Western populations (Chapter 4).

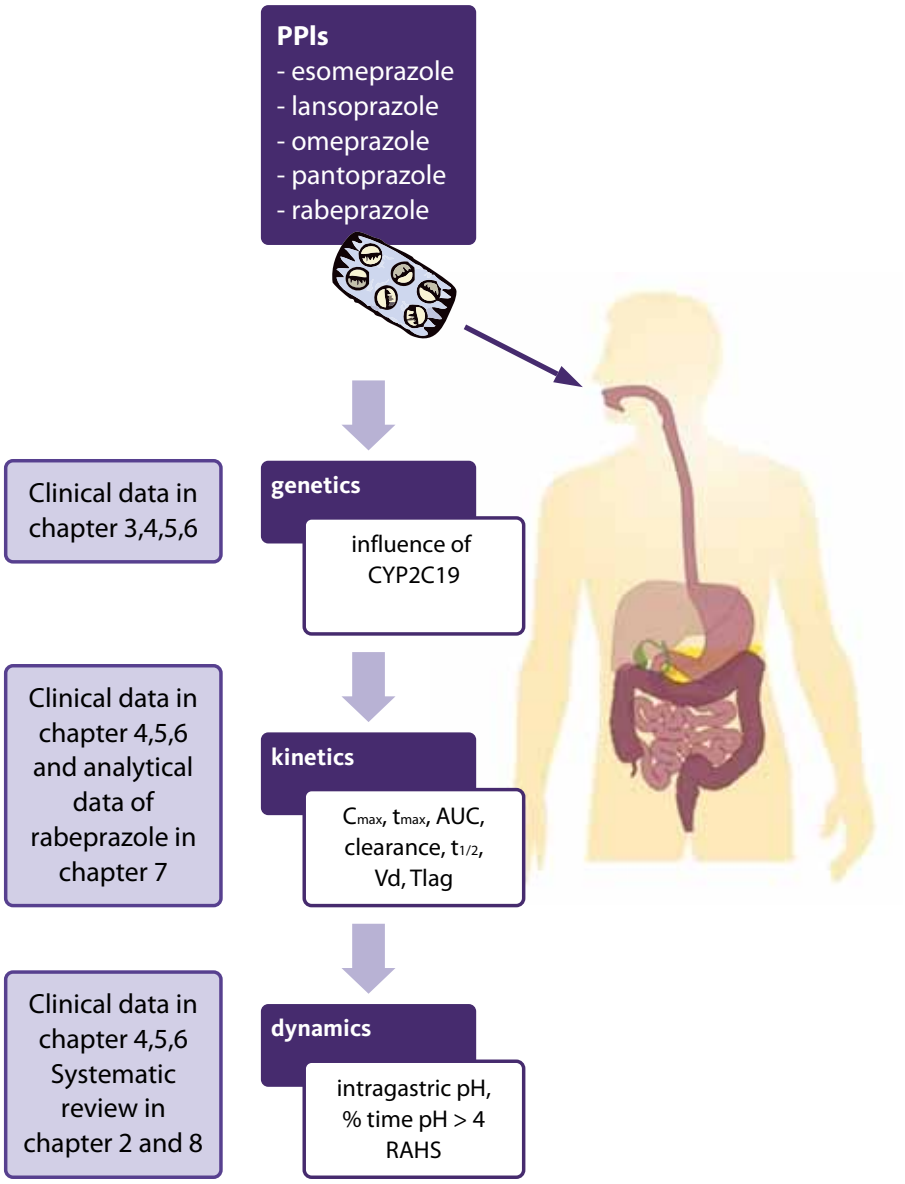
Although there are data about the differences between esomeprazole 40 mg, pantoprazole 40 mg and rabeprazole 20 mg, most studies do not report both pharmacodynamics and kinetics after single administration (day 1) and during steady state (day 5). Furthermore, most studies have not investigated the effect of pharmacogenetic variances. We therefore performed two randomized investigator-blinded cross-over trials. Esomeprazole 40 mg was compared with pantoprazole 40 mg in healthy *H. pylori*-negative subjects after both single and repeated dosing (Chapter 5). And esomeprazole 40 mg was compared with rabeprazole 20 mg in healthy *H. pylori*-negative subjects after both single and repeated dosing (Chapter 6).

The analysis of the PPI rabeprazole in human serum is complicated by the unstable properties of the drug and its long run time. We developed and validated a fast and efficient analysis for the determination of rabeprazole and its metabolite in human serum (Chapter 7).

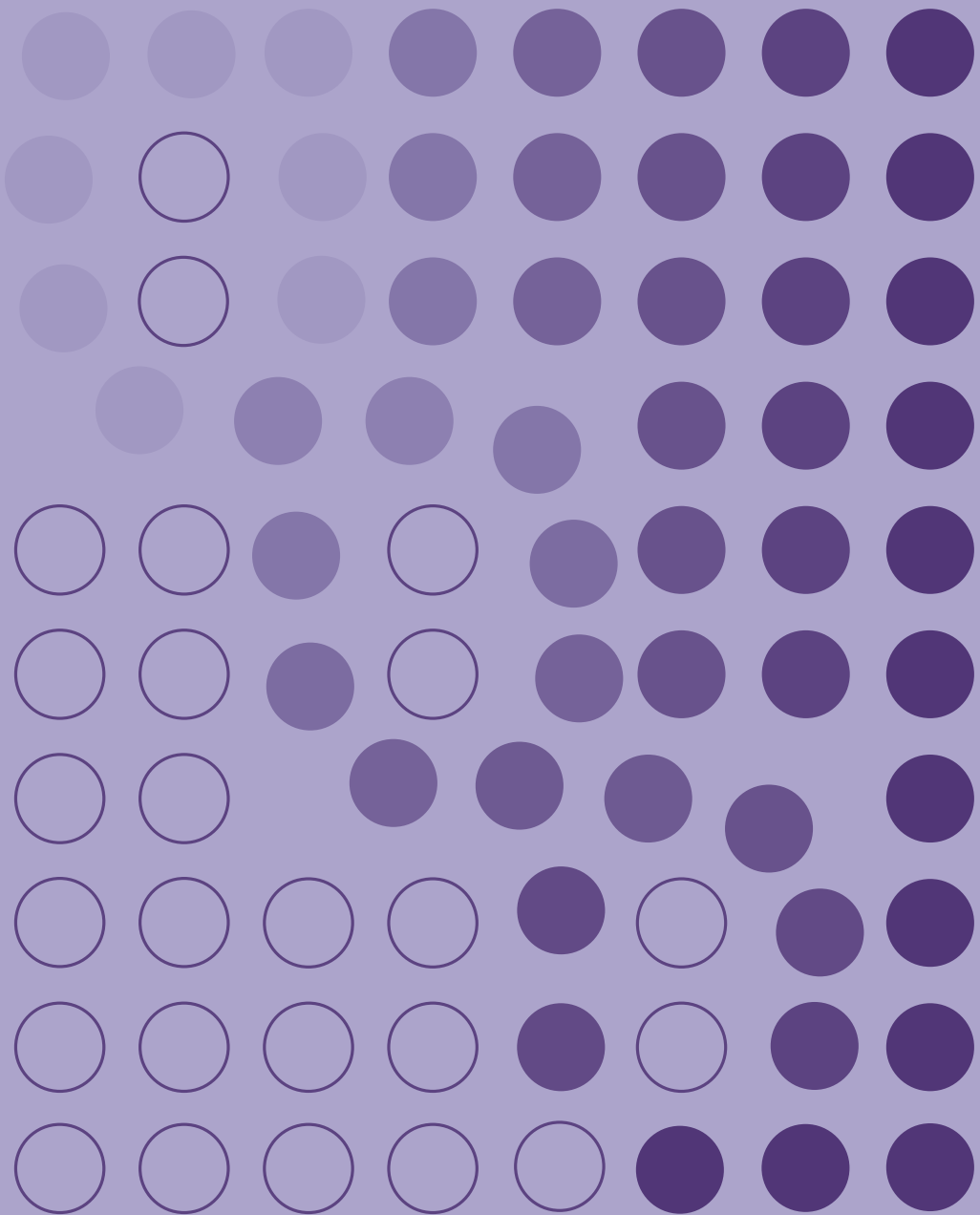
The influence of CYP2C19 on the pharmacodynamics of PPIs is systematically reviewed in chapter 8.

This thesis aimed to answer these questions, thus contributing to the knowledge of pharmacogenetics, kinetics and dynamics of proton pump inhibitors.

Figure 6 Schematic outline of this thesis



Chapter 2



Systematic review: rebound acid hypersecretion after therapy with proton pump inhibitors

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2

2 Systematic review: rebound acid hypersecretion after therapy with proton pump inhibitors

ABSTRACT

Background
The occurrence and the clinical relevance of rebound acid hypersecretion after discontinuation of PPIs is unclear.

Aim
To perform a systematic review of RAHS after discontinuation of PPIs.

Methods
Pubmed, Embase and Central were searched up to October 2005 with indexed terms.

Results
8 studies were included, sample size was 6-32. The studies used both basal and stimulated acid output as parameters to study rebound acid hypersecretion and assessed these at different time points and with variable methods. Five studies (including four randomized studies) did not find any evidence for RAHS after PPI therapy. Of the remaining three studies, the duration of PPI therapy was the longest and two of these studies were the only to assess *H. pylori* status of their study subjects. These two studies suggested that RAHS may occur in *H. pylori*-negatives after 8 weeks of PPIs.

Conclusion
Studies that have investigated RAHS after cessation of PPI treatment are 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. There is no strong evidence for a clinically relevant increased acid production after withdrawal of PPI therapy.

INTRODUCTION

Gastro-oesophageal reflux disease (GERD) is an increasingly common disease with a current estimated prevalence of 20% in Western populations [1, 2]. The main treatment for GERD is acid inhibition with either a PPI or a H_2RA. In the past, it has been shown that discontinuation of H_2RA therapy could lead to rebound acid hypersecretion (RAHS) [3-9]. RAHS is defined as an increase in gastric acid secretion above pre-treatment levels following discontinuation of antisecretory therapy [10]. Because it is conceivable that RAHS contributes to the recurrence of GERD [6], the phenomenon of RAHS is of clinical interest. As PPIs are nowadays the therapy of choice in the management of GERD, the effects on gastric acid production after termination of treatment are relevant. Four mechanisms have been postulated to explain RAHS after treatment with antisecretory drugs: upregulation of H_2-receptors, hypergastrinemia-stimulating histamine release by enterochromaffin-like (ECL) cells, increase of parietal cell mass, and upregulation of H^+/K^+-ATPase activity [3-9, 11-15]. These phenomena play a role during and after H_2RA treatment and have also been claimed to occur at a clinically relevant level after PPI treatment. However, the occurrence and clinical relevance of RAHS after PPI are questionable. Therefore, this systematic review focuses on RAHS after therapy with PPIs.

METHODS

Pubmed, Embase and Central (the database from Cochrane) were searched up to October 2005 for the indexed terms: 'Rebound acid hypersecretion', 'Rebound hypersecretion' and 'Esomeprazole / omeprazole / lansoprazole / pantoprazole / rabeprazole / PPI and hypersecretion'. Published trials evaluating RAHS studied with nasogastric acid aspiration or intragastric pH monitoring were considered for inclusion. Abstracts were excluded. Reviews and studies were rated according to the following rating system [16]: (A1) systematic review containing several studies of A2 level and with consistent outcomes, (A2) prospective randomized-clinical trials of good quality, (B) randomized-clinical trials of moderate quality (e.g. limited patient numbers, impairing strength of the study) or other comparative trials (e.g. not randomized, cohort studies, case-control studies), (C) non-comparative trials, and (D) experts' opinions (e.g. according to the authors).
In addition, a level of evidence was assigned as follows. Evidence that was supported by one systematic review (A1) or at least 2 independent trials of level A2 was graded as level 1, evidence supported by at least 2 independent studies of level B as level 2, evidence supported by one study of level A2, B, or C, as level 3, and evidence that was only supported by experts' opinions was graded as level 4. For each investigated method, the review paragraph is summarized by a conclusion with a level of evidence.

RESULTS

Eight studies were identified that investigated RAHS after PPI treatment (Table 1).
No systematic review has been performed. All studies investigated oral PPI administration. Four studies had a double-blind randomized-clinical design [5, 17-19], the other four trials had an open label design [20-23]. Gastric acid production was measured with different methods and techniques. Studies are grouped below according to the methods and techniques used, in particular aspiration studies with determination of basal acid output, maximal acid output, and peak acid output, aspiration studies with determination of 24 h intragastric acidity or integrated nocturnal acidity, and studies using intragastric pH-monitoring.
Omeprazole was used in seven studies, one study investigated RAHS after discontinuation of lansoprazole therapy. Six studies included healthy subjects, one study was performed in patients with duodenal ulcer (DU) disease, and one study investigated patients with reflux oesophagitis. The median number of subjects per study was 16 (range: 6-32). Three studies included less than 10 subjects [17, 20, 23].

Table 1 Details of reviewed studies

Ref	Study design	*n*	M/F	Subjects	Treatment Daily Dose (mg)	Treatment Duration (days)	A or P	Days to follow-up after discontinuation	HP status assessed	RAHS	Level of evidence
17	RCT, PC	6	M	HV	O 20,40, placebo	1	A	1,3,4,14	No	No	B
18	RCT, DB, PC	16	M	HV	O 40, placebo	14	A	7,14,21,56	No	No	A2
23	Open	9	M	RO	O 40	90	A	14	Yes	Yes	C
21	Open	21 7	9M/12F 4M/3F	HV	O 40	56	A	15 6	Yes	Yes: in HP -	B
22	Open	32	11M/21F	HV	O 40	56	A	7,14,28,42,56	Yes	Yes: (s)MAO in HP - and HP E	B
20	Open	9	M	DU	O 30-60	14	A	7,56	No	No	C
5	RCT, DB, PC	22	M	HV	O 40, placebo, R 300	25	A	3,6,9,12,15,18,21	No	No	A2
19	RCT, DB, PC	16	M	HV	L 30, placebo	14	A and P	2,4,7,14 (P) 2,5 (A)	No	No	A2

HV, healthy volunteers; DU, duodenal ulcer; RO, reflux oesophagitis; O, omeprazole; L, lansoprazole; R, ranitidine; A, aspiration (acid output); P, 24-h continuous pH-monitoring; RCT, randomized-clinical trial; PC, placebo controlled; n, number; M, male; F, female; (s)MAO, (sub)maximal acid output; E, eradicated

Aspiration studies with determination of BAO, MAO, and PAO

Acid secretion can be determined by aspiration. With this method gastric contents are obtained through a nasogastric tube at timed intervals under different test conditions. The hydrogen ion concentration in these samples can be determined providing a quantitative assessment of basal or stimulated acid secretion. Gastric acid secretory tests measure basal acid secretion (BAO) and maximal (MAO) or peak secretory capacity (PAO). BAO is defined as the sum of four 15-minute collections prior to any stimulation. MAO is defined as the sum of the four highest consecutive 15-min or six highest consecutive 10-min periods induced by an optimal dose of stimulant. PAO is defined as the sum of the 2 highest 15-min or 10-min periods within 2 hours of receiving the stimulant, multiplied by 2 or 3 to yield results in mmol H^+ per hour [24].

The first study on RAHS after PPI treatment, performed with aspiration studies was published in 1983 [17]. Six healthy male subjects were given a single dose of omeprazole 20 mg, omeprazole 40 mg, or placebo in a randomized crossover setting. Pentagastrin-stimulated MAO was measured at days 1, 2, 3 and 14 after the single dose of omeprazole or placebo. There was no increase in gastric acid production above pre-treatment levels after termination of therapy. The coefficient of variation was < 10% when the aspiration measurements were repeated with an interval of 24 hours. The *H. pylori*-status of the study subjects was not determined.

In a second study, 16 healthy volunteers were randomized to two weeks of therapy with omeprazole 40 mg or placebo in a double-blind setting [18]. BAO and PAO were measured before, and 1, 2, 3 and 8 weeks after the therapy period. PAO was reproducible with a within-subject variability of 11%, but BAO showed 68% within-subject variability. There was no significant difference in PAO between the two study groups at any of the given time-points. Fasting gastrin concentrations in plasma were determined at each study day. Fasting plasma gastrin levels were elevated during treatment with omeprazole and normalized one week after cessation, except for 3 Asian subjects in whom gastrin levels remained elevated throughout the follow-up period. The *H. pylori*-status of the study subjects was not assessed. This study showed that two weeks of treatment with 40 mg omeprazole once daily did not lead to an increased acid secretory capacity.

In an open study with nine male patients with reflux oesophagitis (assessed by endoscopy), BAO and pentagastrin-stimulated acid secretion were determined before, and 14 days after 90 days of treatment with omeprazole 40 mg. Pentagastrin-stimulated acid secretion was presented as mmol acid output per hour; however the method of aspiration was not specified in the publication. Reproducibility of BAO and pentagastrin-stimulated acid secretion were not tested. Basal as well as meal-stimulated gastrin values were determined before and during treatment. Biopsy samples from the oxyntic mucosa were taken before and at the end of the treatment period for chemical evaluation of ECL cell mass by measurement of histamine and chromogranin A (CgA). The results showed a significant increase in BAO (raw data not presented) and pentagastrin-stimulated acid secretion (an increase from 27.9 to 42.4 mmol/h) after cessation of omeprazole treatment. Three of the nine patients were *H. pylori*-positive, one of them was excluded and the two other patients showed a contradictory effect on BAO and pentagastrin-stimulated acid secretion. No conclusion about the effect of *H. pylori* infection on RAHS could be drawn. The authors found an increase in gastrin, histamine and CgA and hypothesized that a substantial increase in meal-stimulated gastrin release during omeprazole treatment resulted in an increased ECL cell mass [23].

Two open studies in healthy subjects investigated the influence of *H. pylori* on gastric acid secretion after the use of a PPI [21, 22]. The first study assessed the effect of omeprazole on BAO and gastrin-17-stimulated MAO in 12 *H. pylori*-negative and 9 *H. pylori*-positive subjects [21]. They were given omeprazole 40 mg for 8 weeks and BAO and MAO levels were studied before, during, and at day 15 after cessation of therapy. Gastrin levels were determined basally and after infusion of gastrin-17. Reproducibility of BAO and MAO was not tested. At day 15, BAO and MAO were in *H. pylori*-negatives significantly higher than at baseline (BAO increased from 3.0 to 6.8 mmol/h and MAO from 32.4 to 40.4 mmol/h), whereas they did not significantly change in *H. pylori*-positive subjects (BAO from 3.0 to 1.9 mmol/h and MAO from 30.0 to 38.0 mmol/h), even though the actual change of MAO was similar and the proportional change larger in *H. pylori*-positives than in *H. pylori*-negatives. Additionally, the same study evaluated seven other *H. pylori*-negative subjects before, during, and at day 6 after cessation of omeprazole treatment. BAO levels were significantly lower and MAO levels significantly increased at day 6 post-cessation compared with baseline (change in BAO from 3.5 to 1.9 mmol/h and in MAO from 36.0 to 51.7 mmol/h) [21]. Gastrin levels remained stable throughout the study in all subjects.

In a following open study the effect of *H. pylori*-infection on gastric acid production was investigated with pentagastrin [22]. BAO, MAO and sub-maximal acid output (sub-MAO) were measured at baseline and on days 7, 14, 28, and 56 after cessation of an 8-week therapy period with omeprazole 40 mg. The investigators included 12 *H. pylori*-negative, 10 *H. pylori*-positive and 10 *H. pylori*-eradicated subjects. Subjects received eradication treatment during the last 2 weeks of the 8 weeks treatment with omeprazole. Plasma samples for assay of gastrin concentrations were collected after an overnight fast. In *H. pylori*-negative subjects, the BAO was similar to baseline at all time points post-treatment, whereas sub-MAO and MAO were significantly increased at these time points post-treatment. The increase of sub-MAO ranged between 2.3 and 6.9 mmol/h which corresponded with 16-47% of the baseline value. The increase in MAO ranged between 4.5 and 11 mmol/h, corresponding to 16-40% of the baseline value. In *H. pylori*-positive subjects, BAO was markedly increased at day 28, sub-MAO remained unchanged and MAO was increased at days 28 and 42 after cessation of therapy. In *H. pylori*-eradicated, BAO was only increased at day 56. Sub-MAO was increased at all time points and MAO was increased until day 28 after cessation. During treatment, fasting plasma gastrin levels were significantly increased in *H. pylori*-negative and *H. pylori*-positive subjects, but the rise did not reach statistical significance in eradicated subjects. No comparisons were made between the three investigated groups. However, it was remarkable that the *H. pylori*-positives and *H. pylori*-negatives did in fact not differ much with respect to their post-treatment levels, but instead differed most with respect to the pre-treatment MAO levels, being lower in the *H. pylori*-negatives. After cessation of treatment, gastrin levels in all subjects were comparable to pre-treatment levels. The authors concluded that RAHS occurred in *H. pylori*-negative subjects as well as in *H. pylori*-eradicated subjects, but not in *H. pylori*-positives, yet the difference between *H. pylori*-negatives and *H. pylori*-positives in particular seemed to have resulted from a lower pre-treatment MAO in *H. pylori*-negative subjects.

In summary, there is one relatively large study in healthy volunteers with level A2 evidence that did not show RAHS. The *H. pylori* status was not determined in this study. The results of this study are contradicted by two other studies that did show RAHS after cessation of PPI therapy, in particular in *H. pylori*-negatives. However, the latter two studies had an open design, resulting in level C evidence. Furthermore, the results of the studies were contradictory with respect to effect of temporary PPI treatment on BAO. Together, this results in a level 3 evidence for the presence or absence of RAHS after PPI therapy.

Aspiration studies with determination of 24 hr intragastric acidity or integrated nocturnal acidity

Two studies studied RAHS using the aspiration method to measure 24-hr intragastric acidity or integrated nocturnal acidity [5, 20]. The first study was an open study in which nine male DU patients first received 30 mg omeprazole for 2 weeks and then continued for 1 week with either 30 mg (n=4) or 60 mg (n=5) omeprazole [20]. Intragastric 24-h acidity was measured before, during and at 7 and 56 days after cessation. Plasma gastrin levels were determined at 7 and 56 days after cessation. The *H. pylori* status of the study subjects was not determined, however the fact that these were DU patients justifies the assumption that the majority of them was *H. pylori*-positive. Seven days after termination of therapy, acid production was still 26% lower than pre-treatment, while gastrin levels increased from 7.3 ± 1.2 to 18.9 ± 4.4 pM. Fifty-six days after the therapy, 24-h intragastric acidity had returned to the pre-treatment level (pre-treatment: 38.7 ± 3.9 mM, post-treatment 40.3 ± 4.1 mM), showing recovery of normal gastric function combined with normalized gastrin levels.

In the second aspiration study, integrated nocturnal acidity was studied in 24 healthy men [5]. Subjects were randomized in a double-blind, double-dummy design to receive either 300 mg ranitidine at night or 40 mg omeprazole in the morning for 25 days. Intragastric contents were aspirated hourly from 9 PM to 7 AM twice before therapy, at the final day of dosing, and every third night for 21 days after cessation of therapy. Within-subject variability in integrated nocturnal acidity was 7%. *Helicobacter pylori*-status was not determined. In subjects treated with ranitidine, a significant increase in integrated nocturnal acidity was observed on days 3 and 6 after withdrawal of therapy. This was not seen in the omeprazole-treated subjects, in whom integrated nocturnal acidity recovered from hypoacidity to pre-treatment values by day 6 after termination and remained stable thereafter. Gastrin levels in the omeprazole group were only statistically increased on the last day of dosing compared to pre-treatment values.

In summary with respect to aspiration studies with determination of 24-h intragastric acidity or integrated nocturnal acidity, one relatively large study in healthy volunteers with level A2 evidence and one small study in DU patients with level C evidence showed no evidence for RAHS after withdrawal of PPI therapy. This results in a level of evidence of 3 for the absence of RAHS after PPI treatment.

Studies using intragastric pH-monitoring

Intragastric pH monitoring to assess RAHS was used in one study. With this method a catheter containing a single pH electrode is placed trans-nasally into the body of the stomach. The catheter is connected to a computerized module that can digitally record changes in intragastric pH [25]. Sixteen healthy males with undetermined *H. pylori*-status participated in a randomized placebo-controlled double-blind trial [19]. Lansoprazole 30 mg was administered once daily for 14 days. The potential for rebound acidity after the final dose was evaluated by comparing baseline and post-treatment intragastric pH measurements. Furthermore, BAO and pentagastrin-stimulated acid output (parameter not defined) were determined by the aspiration technique. Intragastric pH was monitored during 24-h on days 1, 14, and post-treatment at days 2, 4, 7 and 14. At 2-day post-treatment, there was still an acid-inhibitory effect, but the mean intragastric pH in the lansoprazole-treated group at days 4, 7, and 14 post-treatment did not differ from baseline or from the values found in the placebo-treated subjects. BAO and stimulated acid output were measured before and on day 13 of therapy and post-treatment at days 3 and 5. After withdrawal of lansoprazole treatment BAO and stimulated acid output returned to baseline levels in 2-4 days without any overshoot, indicating the absence of acid rebound. Serum gastrin levels increased significantly during therapy and returned to normal levels in all study subjects within 14 days after treatment. Thus, it can be concluded that there is one report with level A2 evidence which showed that 24-h intragastric pH, BAO and stimulated acid output are not affected by RAHS after cessation of PPI therapy. This results in level 3 evidence for the absence of RAHS after PPI treatment.

DISCUSSION

Proton pump inhibitors are very commonly used for a variety of upper gastrointestinal conditions. In a cohort of approximately 600 000 Dutch subjects, we recently found that annually 4% of the population receives at least one PPI prescription [26]. In the majority of indications, PPIs are prescribed for weeks to months, after which therapy is withdrawn. From these perspectives, the possibility of RAHS after withdrawal of PPI therapy is of clinical relevance. RAHS has been claimed in several publications, but based on this review the overall level of evidence for the occurrence of RAHS can be classified as level 3, or insufficient. This is due to several factors, in particular the large heterogeneity in study design, variable study methods, different study populations, and contradictory results.
The reviewed studies used both basal and stimulated acid output as parameters to study RAHS and assessed these at different time points and with variable methods. Some studies actually investigated an increase of acid production (increased BAO), while others investigated an increase in the capacity to produce acid (determined as MAO or PAO).

Most of the reviewed studies assessed potential RAHS in mixed populations of *H. pylori*-negative and *H. pylori*-positive subjects. The results of these studies were contradictory. Five studies did not find any evidence for RAHS after PPI therapy [5, 17-20]. These five studies included all four randomized studies performed in this field of research [5, 17-19]. However, none of these five studies had assessed the *H. pylori* status of their study subjects. In one study, these subjects all had DU disease and thus were likely to have been infected with *H. pylori* [20]. The remaining four studies only included healthy volunteers, a considerable proportion or possibly the majority of them may have been *H. pylori*-negative [5, 17-19].

In contrast to these five studies, there were three others, all with a non-randomized design, which did report evidence of RAHS [21-23]. Two of these studies were the only to assess *H. pylori* status in their study subjects [21, 22]. These two studies suggested that RAHS may occur in *H. pylori*-negatives, and not in *H. pylori*-positives. This would be explained by the interaction between *H. pylori* colonization and acid production. *Helicobacter 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* [27]. Inflammation of the acid productive region leads to a further reduction of gastric acid production. This is thought to be due to cytokine-induced suppression of parietal cell function, which effect may in the long-term be augmented by a loss of oxyntic cell mass [28, 29].

This hypothesis would provide an explanation for the occurrence of RAHS in *H. pylori*-negatives and not in *H. pylori*-positives [21, 22], but the evidence in favour is for now very limited and further studies are needed. The two studies in *H. pylori*-negative subjects that have demonstrated RAHS showed contradictory results [21, 22]. The first study reported an increase in BAO in *H. pylori*-negative subjects [21], but this could not be confirmed in the second study [22]. Furthermore, both studies reported an increase in MAO in both *H. pylori*-negatives and *H. pylori*-positives [21], the actual change being similar in both groups. The post-treatment MAO levels were comparable in both studies in *H. pylori*-positives and *H. pylori*-negatives.

The conflicting results with respect to post-treatment BAO and increase of MAO observed in these studies are compatible with an increased secretory capacity after withdrawal of PPI therapy, but provide little evidence for an actually increased acid production. Three further studies investigated other parameters than BAO or stimulated acid production, like 24-h intragastric acidity or integrated nocturnal acidity, and those studies did not find evidence of RAHS [5, 19, 20]. However, in these studies, the effect of *H. pylori* status on RAHS was not investigated.

The three studies that observed RAHS [21-23] had treated patients for 56-90 days compared with 1-25 days in the studies that did not show RAHS. It could thus be that occurrence of RAHS is more related to the duration of treatment than to the *H. pylori* status of treated individuals. As there are no further data to support or refute this hypothesis, additional research is needed. We previously observed that 70% of patients who start PPI treatment, stop this therapy within the first year [26]. After the first month, this occurs very gradually over the year. In those who continue treatment, treatment compliance decreases over the first year. Both observations argue against significant rebound effects in daily clinical practice in patients who are treated longer than 56-90 days but do certainly not refute the possibility that RAHS may be related to treatment duration.

What would be a likely explanation for an increased acid secretory capacity after withdrawal of PPI therapy? Various mechanisms have been mentioned in the literature, in particular hypertrophy and hyperplasia of parietal cell mass [22], hypergastrinaemia leading to ECL cell hyperplasia [23], and an increase in either the number of parietal cells or in the expression of H^+/K^+-ATPase mRNA [30, 31]. However, none of these explanations is based on solid evidence. Hypertrophy and hyperplasia of parietal cell mass were not investigated in studies which specifically addressed this issue [21, 22], although parietal cell protrusion, swelling and bulging with protrusion into the glandular lumen can be observed in 80% of PPI users within 3 months of treatment [32]. This phenomenon occurs similarly in *H. pylori*-positives and *H. pylori*-negatives. One small study reported that hypergastrinaemia during PPI therapy indeed led to an increase in ECL cell mass, but evaluation of ECL cell mass by CgA levels in serum was only measured during therapy and not after cessation of PPI treatment. Furthermore, data from *H. pylori*-positive and *H. pylori*-negative subjects were not analyzed separately [23]. For the near future, the ideal design to investigate RAHS after therapy with PPIs would be a study in which *H. pylori*-negative and *H. pylori*-positive subjects are treated with either a placebo or a PPI for at least 56 days in a randomized, investigator blind setting. Basal and stimulated acid output as well as symptoms should be determined before and at days 7, 14, 28, and 56 after cessation of therapy by a well-described aspiration protocol with known reproducibility and variability [33], and statistics should be performed on actual values instead of on proportional changes.

All together, there is little evidence for a significant RAHS in daily clinical practice. This may explain why intermittent use and uneventful withdrawal of PPI therapy are such common phenomena in daily clinical practice [26]. However, clinicians may consider a gradual step-down of PPI treatment in patients who have been treated for longer duration and who have experienced a rapid recurrence of symptoms after previous treatment withdrawal.

CONCLUSIONS

Studies that have investigated RAHS after cessation of PPI treatment are heterogenic in design, methods and outcome. Most studies, including all of those with a randomized design, did not find any evidence for RAHS. Three uncontrolled trials nevertheless suggested an increase in acid secretory capacity in *H. pylori*-negative subjects after 8 weeks of treatment. They did not provide evidence for an increase in basal acid production, but reported that PPI withdrawal may lead to an increase of acid production under conditions of (sub-)maximal stimulation, either in relation to *H. pylori* status or to the duration of treatment. Further studies are needed to clarify these issues. Until now there is 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.

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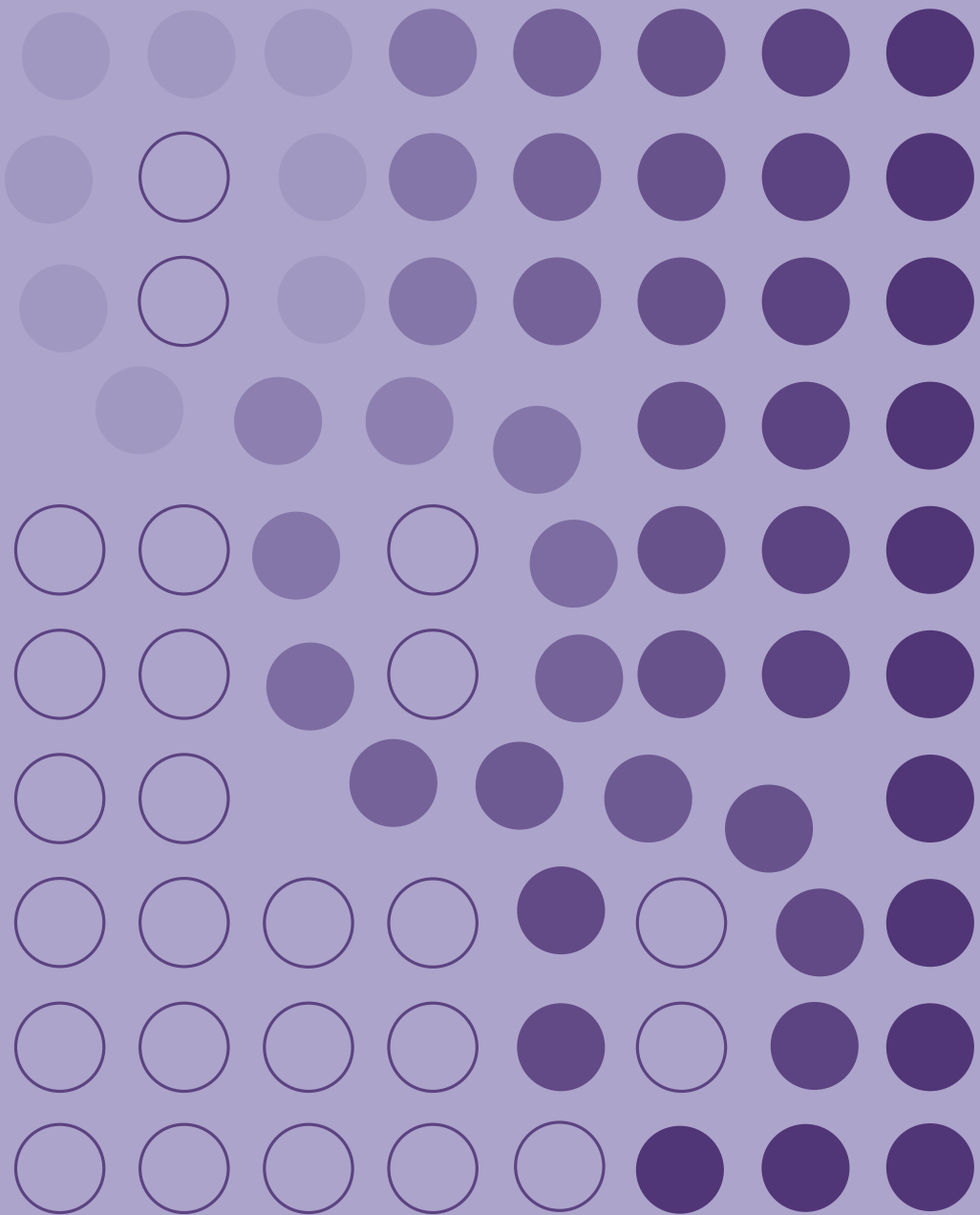
Chapter 3



Genetic polymorphisms of CYP219 in a Dutch population

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ABSTRACT

Background

Variants of CYP2C19 may result in a decreased or increased 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. The presence of these polymorphisms thus impacts the efficacy of drugs which are metabolized by CYP2C19. In the present study we investigated the prevalence of *CYP2C19*2* to **6* and **17* variant alleles in the Dutch population.

Methods

A total of 203 healthy Dutch Caucasian subjects were genotyped for *CYP2C19*2* to **6* and **17* alleles, using PCR-RFLP methods.

Results

The *CYP2C19*2* and **17* allele frequency was both 18%. No **3*, **4*, **5* and **6* alleles were detected. 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 1.5%, respectively.

Discussion & Conclusion

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.

INTRODUCTION

Cytochrome P450 (CYP) 2C19 is an enzyme mediating the metabolism of several important drugs such as diazepam, proton pump inhibitors, proguanil, S-mephenytoin and many anti-depressants [1]. Variants of the CYP2C19 enzyme can results in rapid or poor metabolism of CYP2C19 substrates. *CYP2C19*2* and **3* variants are associated with decreased enzymatic activity. Recently, novel CYP2C19 gene variants have been identified. The **4*, **5* and **6* variants are characterized by respectively an A-->G variant in the initiation codon [2], an Arg433 to Trp substitution in the heme-binding region [3], and a single base pair variant (G395A) in exon 3 resulting in an Arg132-->Gln coding change [4].
These three new variants are also associated with decreased enzymatic activity. In contrast, the fourth novel variant, the **17* allele, is characterized by two SNPs in the promoter region (-3402C>T and -806C>T) and is associated with increased metabolism. In a previous study in the Netherlands, only the prevalence of **2* and **3* variants was studied [5]. The aim of the present study was to determine the prevalence of *CYP2C19*2* to **6* and **17* alleles in the Dutch population.

METHODS

A total of 203 subjects (64 male/139 female) participated in our study. Their mean age was 24 years (range 18-53 years). All participants resided in the Rotterdam-Den Haag area, but originated from all parts of the Netherlands. They completed a questionnaire concerning the ethnic origin of their biological parents and the presence of major chronic diseases. Only subjects with Dutch ethnic origin and without previous medical history were included in our study. The study protocol was approved by the Institutional Review Board of HagaTeaching Hospital. All subjects gave their written informed consent.
CYP2C19 genotyping procedures identifying the wild-type gene, *CYP2C19*1*, and the mutated alleles *CYP2C19*2*, *CYP2C19*3*, *CYP2C19*4*, *CYP2C19*5*, *CYP2C19*6* and *CYP2C19*17* were performed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, by the laboratories of Clinical Chemistry of Haga Teaching Hospital and Erasmus University Medical Center as previously described [6, 7]. Data were calculated using the Statistical Package for Social Sciences (SPSS) version 15.0 (SPSS, Inc. IL, USA).

RESULTS

The number and frequencies of CYP2C19 genotypes and alleles in our study group of 203 Dutch subjects are presented in Table 1. Genotype and allele frequencies were in Hardy-Weinberg equilibrium. *CYP2C19*3* to **6* variants were not detected. The **17* allele was present in 69 subjects and its allele frequency was calculated at 17.73% (95% confidence interval: 14.02- 21.45). A total of 3 subjects carrying the *CYP2C19*17* allele were homozygous (incidence: 1.48%, 95% confidence interval: 0 – 3.14) and 50 were heterozygous carriers (allele frequency: 24.63%, 95% confidence interval:18.70 – 30.56). Sixteen subjects were combined heterozygous for *CYP2C19*2* and **17* alleles (incidence: 7.88%,95% confidence interval: 4.18 – 11.59).

Table 1 Prevalence of CYP2C19 genotypes and alleles in a cohort of healthy Dutch individuals (n = 203, alleles = 406)

Genotypes	No. of individuals	Relative frequency (%)	95% confidence interval
CYP2C19			
*CYPC19*1/*1*	80	39.41	32.69 – 46.13
*CYPC19*1/*2*	51	25.12	19.16 – 31.09
*CYPC19*2/*2*	3	1.48	0 – 3.14
*CYPC19*1/*17*	50	24.63	18.70 – 30.56
*CYPC19*2/*17*	16	7.88	4.18 – 11.59
*CYPC19*17/*17*	3	1.48	0 – 3.14
Alleles	**No. of alleles**		
*CYP2C19*1*	261	64.29	59.62 – 68.95
*CYP2C19*2*	73	17.98	14.24 – 21.72
*CYP2C19*3*	0		
*CYP2C19*4*	0		
*CYP2C19*5*	0		
*CYP2C19*6*	0		
*CYP2C19*17*	72	17.73	14.02- 21.45

DISCUSSION

Information on the presence of CYP2C19 alleles in various populations has started to emerge. The *CYP2C19*2* allelic frequency in our population is 18%. This is somewhat higher than previous findings in Caucasian populations with results ranging from 12.9 to 13.3% [5, 8, 9]. An allele frequency of 25% was observed in Asians [9, 10]. Our data support the conclusion that the allelic frequency for *CYP2C19*3* in Caucasians is low (0 to 1.3%) [5, 8]. The prevalence of these alleles is 0.6% for *4 in the Caucasian population [2]. The frequency of the *CYP2C19*5* alleles is low in Chinese (approximately 0.25% in the Bai ethnic group) and Caucasians (< 0.9%) [3]. For *CYP2C19*6* an allele frequency of about 1.4% was observed in Caucasians [4]. The prevalence of *CYP2C19*17* variants has been reported in healthy Swedes, Greek and in German women (18%, 19.61% and 25.1%) [6, 11, 12]. In Polish patients with peptic ulcer disease a frequency of 26% was reported [13]. Patients with psychiatric disorders in Norway were reported to carry this allele in 21% of cases [14]. We found the allele in 18% of our healthy population, very similar to the frequency reported in Sweden and Greece. Similar results have been obtained in Ethiopia (18%), but lower frequencies in Japanese (1.7%) and Chinese (4%) [6, 15]. In conclusion, the prevalence of *CYP2C19*3*, **4*, **5* and **6* alleles is very low, if present at all, in the Dutch Caucasian population, indicating an allele frequency < 0.3%. In contrast, **2* and **17* alleles are common. The high prevalence 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. This variant should be taken into consideration by clinicians prescribing drugs metabolized by CYP2C19 in the Netherlands.

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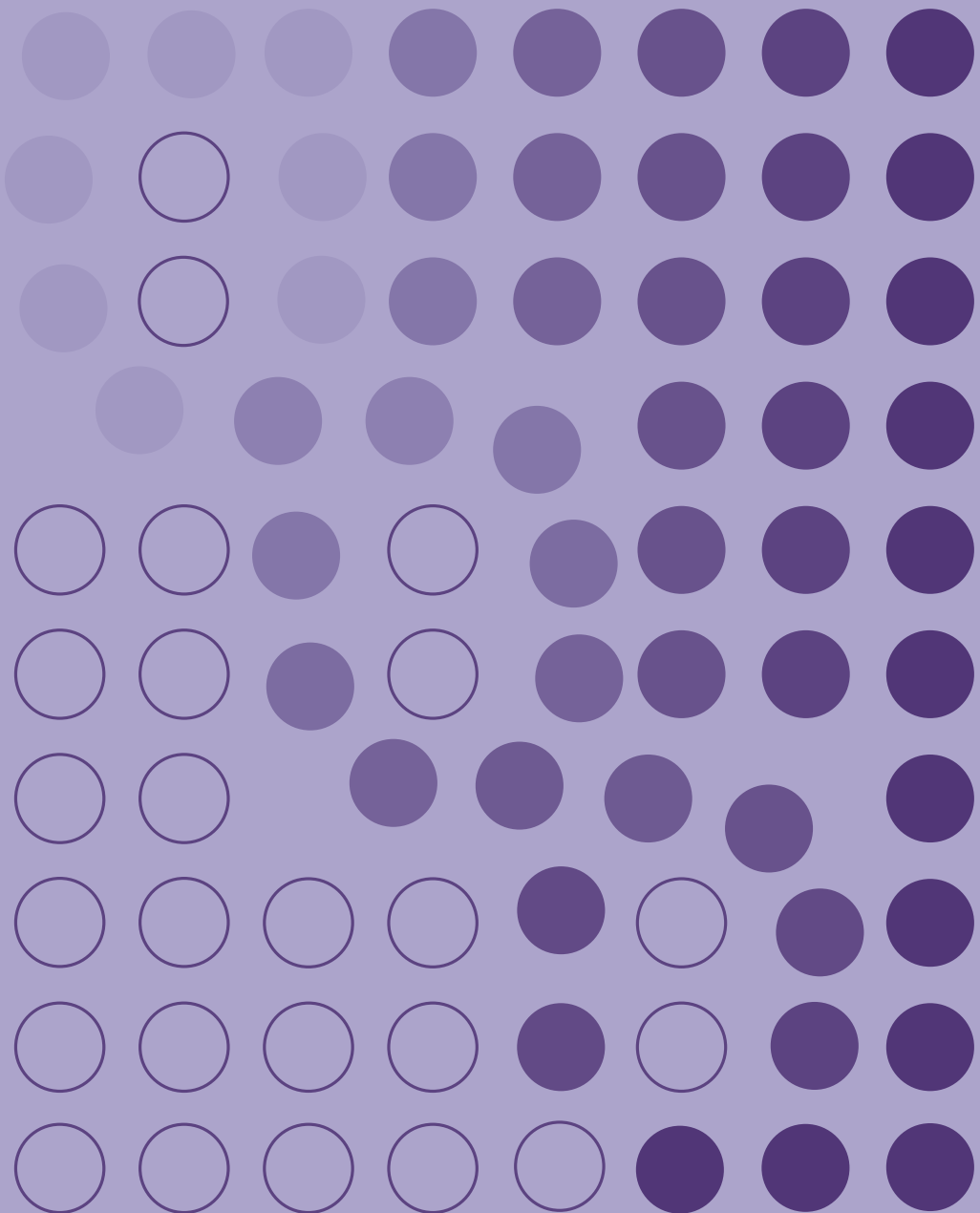
Chapter 4



Effect of *CYP2C19*2* and **17* mutations on pharmacodynamics and kinetics of proton pump inhibitors in Caucasians

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4 Effect of *CYP2C19*2* and **17* mutations on pharmacodynamics and kinetics of proton pump inhibitors in Caucasians

ABSTRACT

Aim

To investigate the impact of CYP2C19 mutations **2-*6* and **17* on acid-inhibition and pharmacokinetics of lansoprazole (L15), omeprazole (O10, O20) and pantoprazole (P40) in Caucasians.

Methods

CYP2C19 genotyping for **2-*6* and **17* mutations 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.

Results

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.

Conclusion

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.

INTRODUCTION

PPIs are metabolized in the liver by the cytochrome P450 system (CYP), specifically CYP2C19 and CYP3A4. Omeprazole for example is mainly metabolized to 5-hydroxyomeprazole by CYP2C19 and to omeprazole sulphone by CYP3A4. CYP2C19 shows genetically determined polymorphisms, which affect the pharmacokinetics and pharmacodynamics of PPIs [1-8].

The genetic basis for the polymorphic expression of CYP2C19 activity has been determined. Several single nucleotide polymorphic variants (SNPs) of the CYP2C19 gene have been identified with impact on the capacity to metabolize PPIs [9]. *CYP2C19*2*, **3*, **4*, **5* and **6* mutations are associated with reduced metabolism of omeprazole, leading to higher AUCs and more profound acid inhibition [9-11]. *CYP2C19*17* mutations are likely to cause increased metabolism of omeprazole, which may result in lower AUCs and reduced acid inhibition [12]. The prevalence of CYP2C19 mutations differs among populations and considering the Eurasian part of the world, an increase in **2* and **3* mutations is seen from West to East. In the Caucasian population about 30-40% has **1/*2* genotype and 2-5% has **2/*2* genotype [13]. In the Chinese population, about 50% has **1/*2* or **1/*3* and 24% has **2/*2*, **2/*3* or **3/*3* genotype. The prevalence of *CYP2C19 *17* mutation is the opposite. About 36% of the Caucasian population has **1/*17* or **17/*17* genotype, about 8% of the Chinese, and about 1% of the Japanese population [12, 14].

Standard doses for the initial treatment of GERD are once daily doses of lansoprazole 30 mg, omeprazole 20 mg, or pantoprazole 40 mg. In many countries the recommended doses for maintenance treatment are once daily doses of lansoprazole 15 mg, omeprazole 10 or 20 mg and pantoprazole 20 or 40 mg. Furthermore, omeprazole 10 mg and 20 mg have been registered in several countries as the first PPIs available over-the-counter. Standard approved doses of PPIs are based on studies performed in subjects with an unknown CYP2C19 genotype [15]. Regarding the current knowledge of pharmacogenetics, it can be speculated that therapy with approved doses of PPIs in Caucasian subjects with fast metabolism (e.g. subjects with **1/*1* genotype or subjects with **17* mutations) could lead to a diminished acid-inhibitory effect and this may result in therapeutic failure.

The aim of this study was to investigate the impact of CYP2C19 mutations **2*, **3*, **4*, **5*, **6* and **17* on the acid-inhibitory effects and pharmacokinetics of lansoprazole, omeprazole and pantoprazole in a Caucasian population.

METHODS

Study protocol

We performed in Caucasian subjects two comparative randomized, two-way cross-over, investigator-blinded studies. In study A the acid-inhibitory effect of lansoprazole 15 mg (L15) was compared with omeprazole 10 mg (O10). In study B the acid-inhibitory effect of omeprazole 20 mg (O20) was compared with pantoprazole 40 mg (P40). To assess the influence of CYP2C19 polymorphism on pharmacodynamics and kinetics of these PPIs CYP2C19 genotype was established in all subjects. In this paper we discuss the effect of CYP2C19 genotype on pharmacodynamics and kinetics.

Both studies were designed to include healthy *H. pylori*-negative subjects whose intragastric pH was below pH 4 for more than 70% of the time during a 24 h baseline period. CYP2C19 genotyping procedures identifying the wild-type gene, *CYP2C19*1*, and the mutated alleles *CYP2C19*2*, *CYP2C19*3*, *CYP2C19*4*, *CYP2C19*5*, *CYP2C19*6* and *CYP2C19*17* were performed by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, by the laboratories of Clinical Chemistry of Haga Teaching Hospital and Erasmus University Medical Center as previously described [12, 16]. The Ethics Committee of Haga Teaching Hospital approved the study protocol.

In study A, the subjects were first assigned to 6 day treatment with either lansoprazole capsules 15 mg once daily or omeprazole capsules 10 mg once daily, followed after a wash-out period of at least 14 days by treatment with the other drug for 6 days. In study B, subjects were assigned to a 6 day treatment with either omeprazole MUPS 20 mg once daily or pantoprazole tablets 40 mg once daily in a similar two-way cross-over design with treatment with the second drug for 6 days after a wash-out period of at least 14 days. In both studies 24-h intragastric pH monitoring took place at day 0 (baseline) prior to drug administration and at days 1 and 6 of administration as previously described [17].

In study A, blood samples (5 mL) for determination of O10 and L15 pharmacokinetics were drawn at day 1 at predose and at 1, 1.5, 2, 3, 4, 6, and 8 h after intake of the study drug.

In study B, blood samples (5 mL) for determination of O20 and P40 pharmacokinetics were drawn at day 1 and day 6 at predose and at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8 and 9 h after dose. Plasma concentrations of study A were determined by means of liquid chromatography techniques at the laboratory of the Central Hospital Pharmacy, The Hague [18, 19]. Plasma concentrations of study B were determined by means of liquid chromatography techniques at the Bio-analytical Chemistry Laboratory, Astra Hässle AB, Mölndal, Sweden [19, 20].

Subjects

Twelve Dutch Caucasian subjects participated in study A and 11 subjects were genotyped (one subject refused genotyping for personal reasons). Sixteen Dutch Caucasian subjects participated in study B and all subjects were genotyped. Subject characteristics and genotypes are shown in Table 1. All subjects gave written informed consent.

Table 1 Subject characteristics and genotypes

	Study A	Study B
All (M/F)*	11 (5/6)	16 (7/9)
**1/*1*↑ (M/F)	5 (3/2)	6 (3/3)
**1/*2* (M/F)	4 (1/3)	2 (0/2)
**1/*17* (M/F)	1 (1/0)	6 (3/3)
**2/*2* (M/F)	0	1 (1/0)
**2/*17* (M/F)	1 (0/1)	1 (0/1)
Age (years) mean (range)	24.2 (20-29)	24.7 (21.4-30)
Weight (kg)mean (range)	70 (50-90)	73 (55-97)
Length (cm) mean (range)	174 (157-190)	176 (157-192)

**(M/F= male/female).* ↑ **3, *4, *5 or *6 mutations were not found*

Data analysis and statistical evaluation of pH data

Evaluation of pH data was performed as previously described [17]. Cumulative percentages of time during which intragastric pH was above 4 for 24-h time periods, was compared between baseline data and the studied PPIs at days 1 and 6 for the total group and for the subgroups with a specific CYP2C19 genotype. 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. A change in this Δ percentage 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 [21]. We defined individuals showing a Δ of ≥10% as responders and individuals with a Δ of < 10% as nonresponders.

Data were analyzed using the SPSS statistical package (SPSS 12.0.1 for Windows, SPSS Inc, Chicago, USA). In various genotype groups the data were too scarce for performing nonparametric tests. Therefore parametric tests were used: paired-samples *t*-test for testing changes from baseline at day 1 and day 6 and independent-samples *t*-test for comparison of these changes between genotypes. However, with scarce data these *t*-tests lean heavily on the assumption of a normal (Gaussian) distribution of the changes from baseline considered. In order to enhance the plausibility of this assumption, a logit transformation of the cumulative percentage of time with pH above the threshold 4 was made prior to calculating changes from baseline and performing *t*-tests. If x denotes the cumulative percentage of time with pH above threshold 4, then the logit of x is defined as the (natural) logarithm of the odds: log(x / (100 – x)). The logit transformation is an appropriate variance-stabilizing transformation for proportions.

As it should, test results based on it would remain the same, when pH-levels below threshold 4 would have been used for calculating the percentage of time. Mean changes from baseline and their confidence limits on the logit scale can be back-transformed by exponentiation, yielding the odds ratios with their confidence limits. The significance level of each test was set at 0.05. Two-sided *P* values were presented as calculated with each test, no correction being made for multiple testing.

Pharmacokinetic data

Pharmacokinetic parameters shown as clearance/*F* (Cl/*F*, in l h^{-1}, *F* is bioavailability), half life ($t_{1/2}$, in h), time of maximum observed concentration (t_{max}, h), and the maximum observed concentration (C_{max}, mg l^{-1}) were derived by noncompartmental analysis using WinNonlin software (version 5.1, Pharsight Corporation, Mountain View, USA). For each individual the terminal elimination rate constant (*k*) was determined by log-linear regression of the terminal phase of the plasma concentration-time curve separately on day 1 (study A and B) and day 6 (study B). The area under the concentration-time curve (AUC; in mg l^{-1} h) was estimated by the linear-logarithmic trapezoidal method up to the last measured data point with extrapolation to 24 h using *k*. Differences between genotypes were evaluated using the independent-samples *t*-test. The level of significance was set at 0.05.

RESULTS

Pharmacokinetics

Differences in AUCs between the genotypes in study A and B are displayed in Table 2 and in Figure 1.

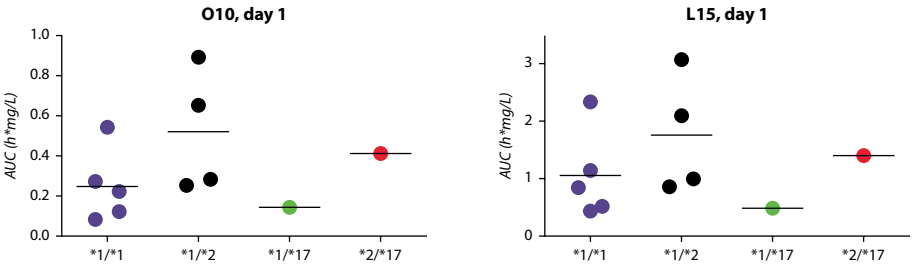
Table 2 AUC (mean values ± SD) at day 1 (Study A and B) and day 6 (Study B) for genotypes

Study A AUC (mg l^{-1} h)	****1/*1*** **(n=5)**	****1/*2*** **(n=4)**	****1/*17*** **(n=1)**	****2/*17*** **(n=1)**	**P value** ****1/*1* vs. **1/*2***
O10 Day 1	0.25 ± 0.18	0.52 ± 0.31	0.14	0.41	0.141
L15 Day 1	1.04 ± 0.77	1.75 ± 1.05	0.47	1.39	0.282

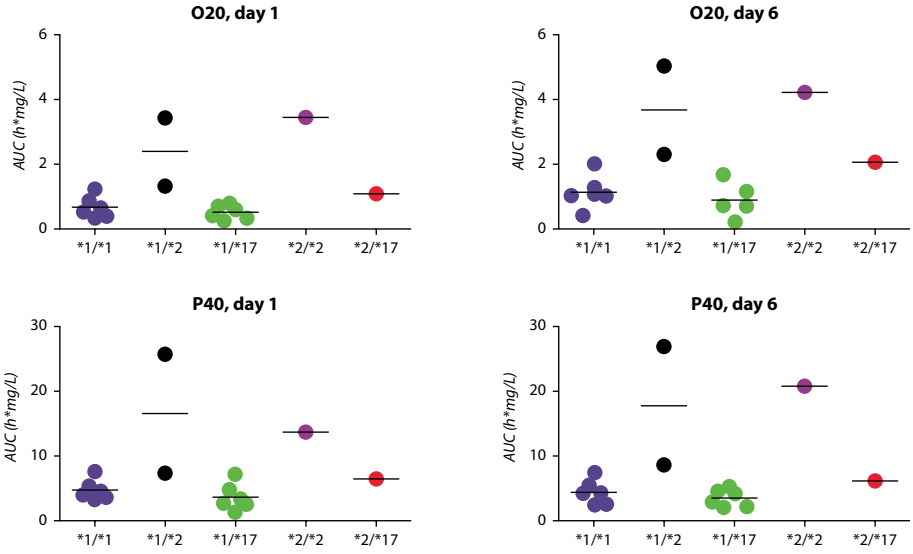
Study B AUC (mg l^{-1} h)	****1/*1*** **(n=6)**	****1/*2*** **(n=2)**	****1/*17*** **(n=6)**	****2/*2*** **(n=1)**	****2/*17*** **(n=1)**	**P value** ****1/*1* vs. **1/*17***
O20 Day 1	0.64 ± 0.34	3.42; 1.30	0.49 ± 0.22	3.44	1.06	0.365
O20 Day 6	1.11 ± 0.52	5.04; 2.29	0.86 ± 0.56	4.22	2.03	0.465
P40 Day 1	4.56 ± 1.60	25.72; 7.16	3.42 ± 2.10	13.56	6.29	0.314
P40 Day 6	4.21 ± 1.91	26.87; 8.49	3.32 ± 1.33	20.71	5.95	0.374

Figure 1 Individual and mean (vertical bar) AUC of **1/*1*, **1/*2*, **1/*17*, **2/*2* and **2/*17* genotypes after administration of L15, O10, O20 and P40 on day 1 (study A and B) and day 6 (study B)

Study A



Study B



For all studied PPIs, the same pattern was seen between genotype and AUC on day 1 and on day 6 with AUC being highest in **2/*2*, and lowest in **1/*17*. Differences between AUCs of **1/*1* and **1/*2* in study A and between **1/*1* and **1/*17* in study B were not significant (all P values ≥ 0.14). The clearance showed the same genotypic trend as the AUC with lowest clearance in **2/*2* and highest in **1/*17* (data not displayed). The **2* and **17* mutations did not influence the pharmacokinetic parameters t1/2, tmax and Cmax (data for total group shown in Table 3).

Table 3 Pharmacokinetic parameters (mean values ± SD) at day 1 (Study A and B) and day 6 (Study B) for total group

	t_{max} (h)	C_{max} (mg l^{-1})	Cl/F (l h^{-1})	$t_{1/2}$ (h)
O10 Day 1	1.55 ± 0.63	0.18 ± 0.11	72.39 ± 61.73	1.21 ± 0.11
L15 Day 1	1.29 ± 0.27	0.40 ± 0.17	32.05 ± 10.09	1.72 ± 0.99
O20 Day 1	1.48 ± 1.38	0.46 ± 0.27	36.10 ± 24.06	1.33 ± 1.46
O20 Day 6	1.33 ± 1.23	0.75 ± 0.31	24.73 ± 28.17	1.15 ± 0.58
P40 Day 1	1.78 ± 1.19	2.85 ± 0.90	10.73 ± 8.29	1.45 ± 0.65
P40 Day 6	2.14 ± 1.90	2.92 ± 0.88	10.40 ± 6.21	1.59 ± 1.17

Acid-inhibition at day 1

Cumulative mean percentage of time with intragastric pH > 4 (± 1 SD) at baseline and during day 1 for the four treatment regimens of the total group and of each genotype are shown in Table 4.

Table 4 Mean percentage of time (± SD) with intragastric pH > 4 during 24 h for total group and genotypes

Study A	**all (n = 11)**	***1/*1* (n = 5)**	***1/*2* (n = 4)**	***1/*17* (n = 1)**	***2/*17* (n = 1)**
Baseline	13.2 ± 7.4	14.9 ± 9.9	11.9 ± 6.3	13.9	9.5
L15 Day 1	34.2 ± 17.3 (^*P* = 0.002)	35.6 ± 21.1[1]	38.3 ± 16.4	15.9	29.7
L15 Day 6	44.2 ± 15.0 (^*P* < 0.0005)	43.1 ± 20.1[2]	49.4 ± 6.8	26.7	46.1
O10 Day 1	22.4 ± 10.9 (^*P* = 0.042)	19.1 ± 10.1[3]	29.7 ± 11.5	12.3	19.5
O10 Day 6	40.3 ± 20.9 (^*P* = 0.006)	36.0 ± 20.0[4]	46.8 ± 18.8	11.5	64.9

^P values: compared with baseline.

*[1] P value *1/*1 vs. *1/*2 after logit-transformation: 0.627, [2]: 0.602, [3]: 0.289, [4]: 0.532*

Study B	**all (n = 16)**	***1/*1* (n = 6)**	***1/*2* (n = 2)**	***1/*17* (n = 6)**	***2/*2* (n = 1)**	***2/*17* (n = 1)**
Baseline	13.5 ± 6.1	14.0 ± 5.5	10.7; 15.3	14.5 ± 8.1	6.1	13.0
O20 Day 1	27.9 ± 16.3 (^*P* = 0.003)	20.4 ± 8.9[1]	48.1; 62.5	21.5 ± 13.4	35.3	48.6
O20 Day 6	51.3 ± 16.6 (^*P* < 0.0005)	50.2 ± 21.0[2]	62.2; 61.9	47.3 ± 17.3	47.7	62.0
P40 Day 1	31.4 ± 14.6 (^*P* = 0.001)	31.5 ± 10.3[3]	50.9; 55.8	20.9 ± 12.8	43.7	37.4
P40 Day 6	47.8 ± 16.4 (^*P* < 0.0005)	44.5 ± 11.9[4]	68.1; 62.0	46.1 ± 20.8	59.8	30.3

^P values: compared with baseline

*[1] P value *1/*1 vs. *1/*17 after logit-transformation: 0.981, [2]: 0.822, [3]: 0.204, [4]: 0.900*

Odds ratios with 95% CI and *P* values (compared with baseline) are shown in Table 5. Δ percentage intragastric pH > 4 for each subject and genotype is shown in Figure 2. Compared with baseline and not differentiating for genotype, L15, O10, O20 and P40 significantly increased the mean percentage of time with intragastric pH above 4 (all *P* values 0.042 or less).

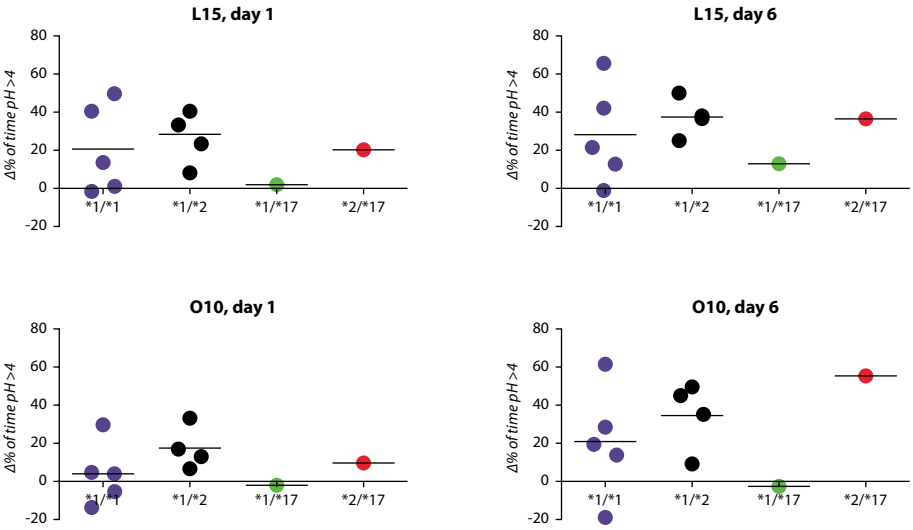
Table 5 Odds ratios (range 95% CI) indicating changes from baseline and *P* values

Study A	***1/*1** **(n=5)**	***1/*2** **(n=4)**
L15 Day 1	3.25 (0.63-16.64) (*P* = 0.116)	4.73 (1.52-14.70) (*P* = 0.022)
L15 Day 6	5.14 (0.85-31.02) (*P* = 0.065)	7.89 (2.87-21.71) (*P* = 0.007)
O10 Day 1	1.49 (0.33-6.67) (*P* = 0.497)	3.24 (1.15-9.12) (*P* = 0.036)
O10 Day 6	3.54 (0.39-31.86) (*P* = 0.185)	6.75 (1.61-28.37) (*P* = 0.024)

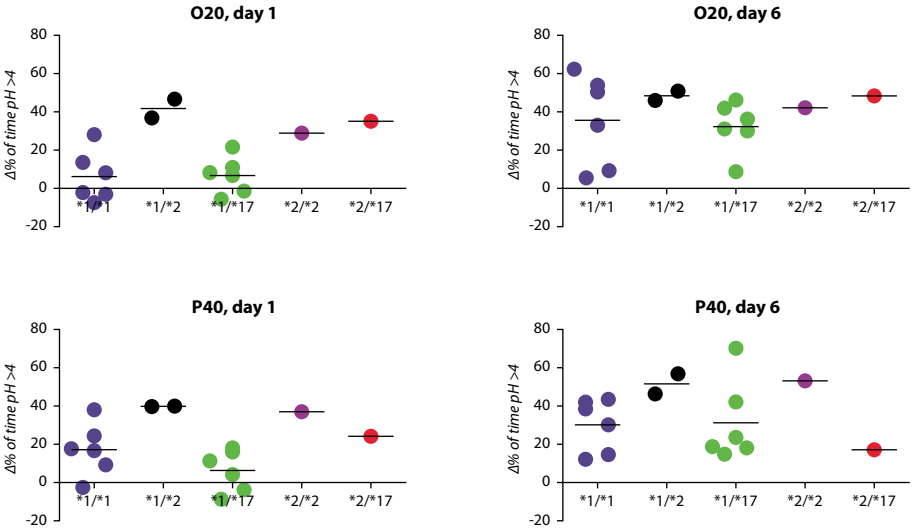
Study B	***1/*1** **(n=6)**	***1/*17** **(n=6)**
O20 Day 1	1.59 (0.54-4.66) (*P* = 0.315)	1.61 (0.87-2.98) (*P* = 0.102)
O20 Day 6	6.66 (1.75-25.32) (*P* = 0.015)	5.82 (2.99-11.32) (*P* = 0.001)
P40 Day 1	2.96 (1.13-7.75) (*P* = 0.034)	1.51 (0.66-3.47) (*P* = 0.258)
P40 Day 6	5.32 (2.29 -12.37) (*P* = 0.004)	5.70 (1.88-17.26) (*P* = 0.010)

Figure 2 Individual and mean (vertical bar) responses of **1/*1*, **1/*2*, **1/*17*, **2/*2* and **2/*17* genotypes to L15, O10, O20 and P40 on day 1 and day 6, corrected for baseline (Δ percentage of time pH > 4)

Study A



Study B



Differentiating for genotype, the **1/*1* genotype showed no significant acid-inhibitory effect after a single dose of L15 ($P = 0.116$), O10 ($P = 0.497$) and O20 ($P = 0.315$). In subjects with **1/*17* genotype, no significant acid-inhibitory effect was seen after a single dose of O20 ($P = 0.102$) and P40 ($P = 0.258$). Only with P40 was there a significant acid-inhibitory effect after a single dose in **1/*1* subjects ($P = 0.034$). Subjects with the **1/*2* genotype showed a significant acid-inhibitory effect after L15 ($P = 0.022$) and O10 ($P = 0.036$). In both studies **1/*1* and **1/*17* genotypes showed lower responses than **1/*2*, **2/*17* or **2/*2* genotypes. However, either the differences between **1/*1* and **1/*2* for L15 and for O10 as well as the differences between **1/*1* and **1/*17* for O20 and for P40 were not significant (all P values ≥ 0.204) or the numbers were too small to test the differences. Table 6 shows the percentage of subjects with a response (Δ percentage of acid-inhibition ≥ 10%) to the administered PPI.

Table 6 Number and percentage of subjects with an acid-inhibitory response of ≥ 10%

PPI	all	**1/*1*	**1/*2*	**1/*17*	**2/*2*	**2/*17*
Study A	*n = 11*	*n = 5*	*n = 4*	*n = 1*	-	*n = 1*
L15						
Day 1	7 (64%)	3 (60%)	3 (75%)	0 (0%)		1 (100%)
Day 6	10 (91%)	4 (80%)	4 (100%)	1 (100%)		1 (100%)
O10						
Day 1	5 (45%)	1 (20%)	3 (75%)	0 (0%)		1 (100%)
Day 6	8 (73%)	4 (80%)	3 (75%)	0 (0%)		1 (100%)
Study B	*n = 16*	*n = 6*	*n = 2*	*n = 6*	*n = 1*	*n = 1*
O20						
Day 1	8 (50%)	2 (33%)	2 (100%)	2 (33%)	1 (100%)	1 (100%)
Day 6	13 (81%)	4 (67%)	2 (100%)	5 (83%)	1 (100%)	1 (100%)
P40						
Day 1	11 (69%)	4 (67%)	2 (100%)	3 (50%)	1 (100%)	1 (100%)
Day 6	16 (100%)	6 (100%)	2 (100%)	6 (100%)	1 (100%)	1 (100%)

Acid-inhibition at day 6

Cumulative mean percentage of time with intragastric pH > 4 (± 1 SD) at baseline and during day 6 for the four treatment regimens of the total group and of each genotype are shown in Table 4. Odds ratios with 95% CI and P values (compared with baseline) are shown in Table 5. Δ percentage intragastric pH > 4 for each subject and genotype is shown in Figure 2. Compared with baseline and not differentiating for genotype, the mean percentage of time with an intragastric pH above pH 4 was significantly increased in all subjects with all four regimens studied (all P values ≤ 0.006). Differentiating for genotype, **1/*1* and **1/*17* showed a significantly increased percentage of time with intragastric pH above 4 after treatment with O20 ($P = 0.015$, resp. $P = 0.001$) and P40 ($P = 0.004$ and 0.010 respectively). However, in **1/*1* subjects treated with L15 and O10 this percentage of time was not significantly increased ($P = 0.065$, resp. $P = 0.185$). The **1/*2* genotype showed a significant acid-inhibitory effect after repeated dosing with L15 ($P = 0.007$) and O10 ($P = 0.024$). No significant difference between **1/*1* and **1/*2* for L15 and for O10 and between **1/*1* and **1/*17* for O20 and P40 was seen was seen at day 6 (all P values 0.532 or more). Table 6 shows the percentage of subjects with a response (Δ percentage of acid-inhibition >10%) to the administered PPI.

DISCUSSION

The aim of the study was to examine the influence of CYP2C19 mutations on the acid-inhibitory effects and pharmacokinetics of lansoprazole, omeprazole and pantoprazole in a Caucasian population. The study showed an effect of CYP2C19 polymorphism on the pharmacodynamics of standard dose pantoprazole, low dose lansoprazole, and low/standard dose omeprazole. This effect was not supported by pharmacokinetic data, probably due to the fact that the power of the studies was based on the comparison omeprazole vs. lansoprazole and omeprazole vs. pantoprazole. Further studies are needed to give a decisive answer on the significance of CYP2C19 polymorphism in Caucasians.

Genotypic analysis of the subjects in study A demonstrated 45% **1/*1*, 36% **1/*2*, 9% **1/*17*, 0% **2/*2* and 9% **2/*17* mutations. Genotypic analysis of the subjects in study B showed 37.5% **1/*1*, 12.5% **1/*2*, 37.5% **1/*17*, 6% **2/*2* and 6% **2/*17* mutations. During genotyping for **3*, **4*, **5* and **6* mutations we did not find any of these mutations in our population [11]. It is known that **3* mutation mainly occurs in Asian subjects. The allelic frequency in our studies reflected the Western genotypes with a slight under representation of the **1/*2* genotype, which is reported to occur in 30-40% of the Western population.

Irrespective of genotype, L15, O10, O20 and P40 produced significant acid inhibition after a single dose and all PPIs studied produced significant acid reduction after repeated dosing.

We have shown in Caucasian subjects with **1/*1* genotype, that on the first day of administration the acid suppression with lansoprazole 15 mg and omeprazole 10 or 20 mg is not significant. In contrast to lansoprazole 15 mg and omeprazole 10 mg, acid suppression with omeprazole 20 mg reached significance after repeated dosing. Only pantoprazole 40 mg showed significant acid-inhibition in **1/*1* subjects after both single and repeated administration. However, in **1/*17* subjects there was no significant acid-inhibitory effect after single administration. At day 6 of administration the acid-inhibitory effect of pantoprazole 40 mg reached significance in **1/*17*.

Omeprazole is the only PPI known to have auto-inhibition of its metabolism [22]. Our study showed that for **1/*1* subjects in contrast to pantoprazole 40 mg, clearance for omeprazole 20 mg was reduced resulting in an increased AUC on day 6 compared with day 1. The increased AUC and the pharmacological steady state explain the more potent inhibition of gastric acid production after repeated dosing with omeprazole. Our pharmacodynamic data nicely illustrate that in **1/*1* subjects a dose of 10 mg omeprazole is too low to benefit from this effect.

Regarding **2* mutations, our data keeping with the findings from Japanese studies that **2* mutations lead to increased AUC and more profound acid inhibition of PPIs [9]. With L 15 mg, significant acid-inhibition was seen in **1/*2* subjects after single as well as after repeated administration. The same occurs with O10. It can be concluded that in **1/*2* subjects, compared with **1/*1* subjects, metabolism of omeprazole is already reduced resulting in a significant acid-inhibitory effect after a single dose. Due to auto-inhibition of its metabolism after repeated dosing, a dose as low as 10 mg will lead to a further increase of intragastric pH.

The novel **17* mutation is associated with therapeutic failure of PPIs [12]. This assumption is based on a decreased metabolic ratio of omeprazole in Caucasian subjects with the **17* genotype. Our study in subjects treated with omeprazole and pantoprazole demonstrated that a **17* mutation may lead to less acid-inhibition and a decreased AUC compared with **1/*1* genotypic subjects. In contrast to the findings in **1/*1* subjects, we found no significant acid-inhibitory effect in **1/*17* subjects after single administration of P40. Repeated administration of pantoprazole showed significant acid-inhibition for **1/*17* subjects with unchanged AUC compared with single administration. This demonstrated that the increase in acid-inhibitory effect after repeated dosing of pantoprazole was caused by a pharmacodynamic effect (reaching a pharmacological steady state) rather than a kinetic effect (c.f. omeprazole).

The majority (e.g. up to 70%) of the Caucasian population has the **1/*1* or **1/*17* genotype and only 30% to 40% has a **2* mutation. In the Asian population however, **2*, and **3* mutations are seen with an allelic frequency up to 75%. In contrast to **1/*1* or **1/*17* mutation, **2*, and **3* mutations are associated with decreased metabolism of PPIs. The differences in pharmacokinetics between subjects with **1/*1* or **1/*17* genotypes on the one hand and subjects with **1/*2* genotypes on the other hand, explains another finding of our study, i.e. that omeprazole 10 or 20 mg and lansoprazole 15 mg have no significant acid-suppressive effect after a single dose in these genotypes as well as pantoprazole in subjects with the **1/*17* genotype. Subjects with **1/*1* or **1/*17* genotypes may need higher doses of PPIs or drugs that inhibit CYP2C19 or CYP3A4 metabolism (e.g. some macrolides) to reach the same acid-inhibitory effect as we found in subjects with **2* mutations. The difference in occurrence of **2* and **17* mutations in Asian and Caucasian populations makes it difficult to extrapolate results found in studies performed in Japanese and Chinese populations to the Caucasian population.

A considerable proportion of our subjects, mainly with **1/*1* and **1/*17* genotype, did not show a more than 10% gain in the proportion of time that intragastric pH was above 4 in a 24-h period. Even with a single dose of pantoprazole 40 mg, 31% of the subjects did not reach this criterion. We think that this criterion is a pertinant parameter for clinically relevant acid-inhibition, given the accuracy of the technique of intragastric pH monitoring and the variability in 24-h intragastric acidity [21]. With all studied PPIs, the number of nonresponders decreased substantially after repeated administration.

CONCLUSION

This study showed that the acid-inhibitory effects of lansoprazole, omeprazole and pantoprazole in Caucasians are influenced by CYP2C19 status. Due to this effect, single and repeated administration of omeprazole 10 mg and lansoprazole 15 mg in **1/*1* subjects did not provide significant acid-inhibition when compared with baseline. After a single dose, acid-inhibition in **1/*1* or **1/*17* subjects with omeprazole 20 mg is not significant, but became significant after repeated administration. Pantoprazole 40 mg provided significant acid-inhibition in **1/*1* subjects but not in **1/*17* subjects after a single dose. After repeated dosing pantoprazole 40 mg showed significant acid inhibition in **1/*17* subjects as well. Because of a remarkably lower (and often inadequate) acid-inhibitory effect in subjects with **1/*1* and **1/*17* genotype for CYP2C19, who comprise together up to 70% of the Caucasian population, stronger acid-suppression therapy needs to be considered, especially during the first days of therapy or with on-demand therapy.

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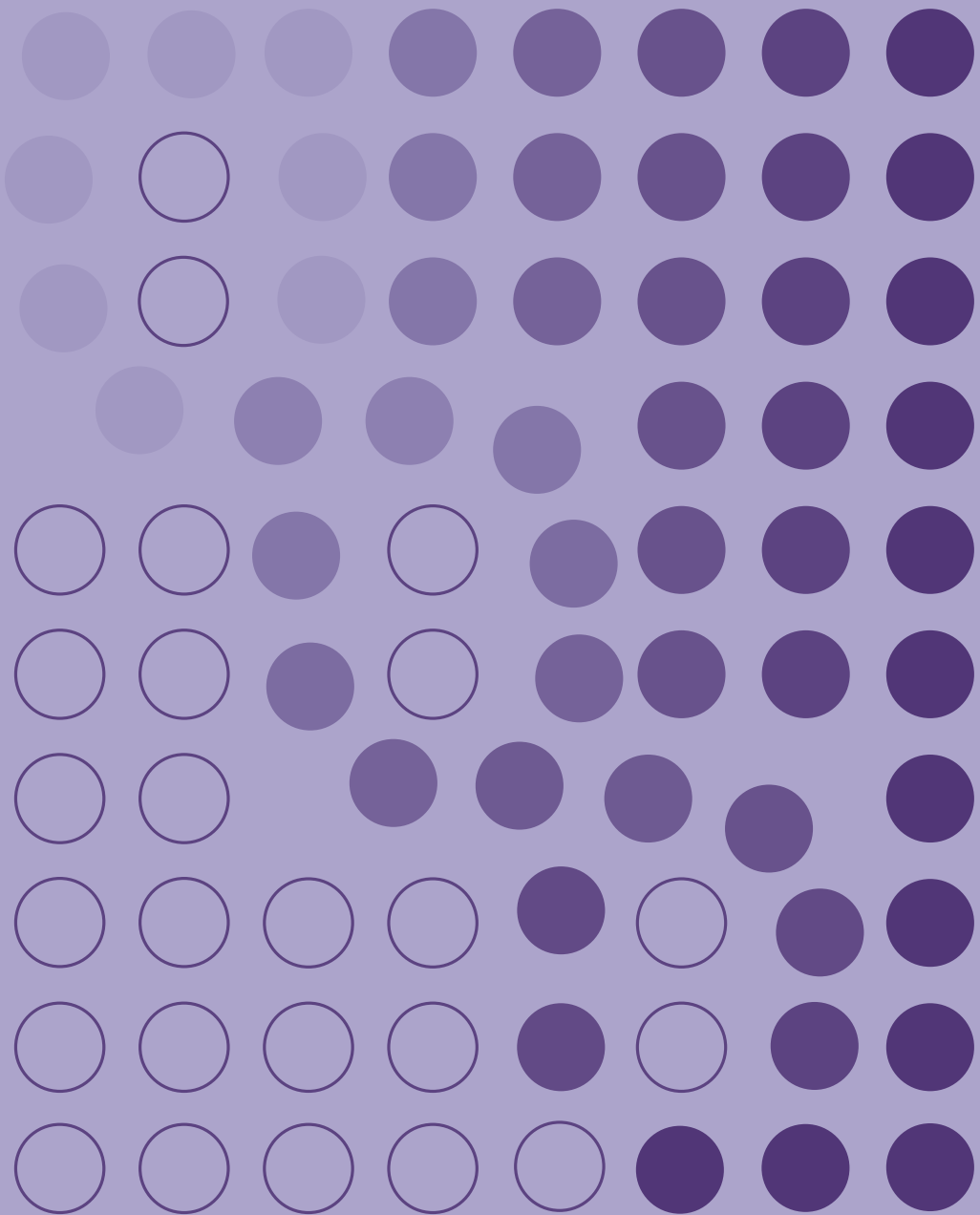
Chapter 5



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

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5

5 A comparison of the acid-inhibitory effects of esomeprazole and pantoprazole in relation to pharmacokinetics and CYP2C19 polymorphism

ABSTRACT

Background
Esomeprazole and pantoprazole are metabolized in the liver and the polymorphic CYP2C19 enzyme is involved in that process. This genetic polymorphism determines fast (70% of Caucasians), intermediate (25-30% of Caucasians) and slow (2-5% of Caucasians) metabolism of PPIs.

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

Methods
*CYP2C19*2*, **3*, **4*, **5*, **6* and **17* genotypes were determined in healthy *Helicobacter pylori*-negative Caucasian subjects. Seven *wt/wt*, seven *wt/*2*, two *wt/*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. Intragastric 24-h pH-monitoring was performed on days 0, 1 and 5 of oral dosing.

Results
19 subjects (mean age 24y, 7 male) completed the study. At day 1 and 5, acid-inhibition with esomeprazole was significantly greater and faster than with pantoprazole. Differences in acid-inhibition and pharmacokinetics between *wt/wt* and *wt/*2* genotype were significant for pantoprazole at day 1 and 5.

Conclusions
Esomeprazole provides acid-inhibition faster than and superior to pantoprazole after single and repeated administration. The acid-inhibitory effect and the kinetics of pantoprazole are influenced by CYP2C19 genotype.

INTRODUCTION

The key to effective management of GERD is provided by extensive and prolonged suppression of gastric acid secretion. Mucosal healing of erosive oesophagitis is directly correlated with the amount of time in a 24-h period that intragastric pH is above pH 4 [1, 2]. PPIs effectively control intragastric acidity during a 24-h period and maintain intragastric pH longer above 4 than H_2-receptor antagonists [3].

However, while all PPIs are effective acid-suppressive drugs, there are pharmacological differences among PPIs. For example, oral doses of esomeprazole (20 or 40 mg) maintain intragastric pH significantly longer above 4 than oral doses of omeprazole (20 or 40 mg) [4, 5], and the acid-suppressive effects of omeprazole 20 mg and pantoprazole 40 mg are not significantly different [6].

Furthermore, it has been shown that the acid-suppressive effects of PPIs are affected by hepatic cytochrome P450 (CYP) enzyme polymorphisms. PPIs are mainly degraded in the liver via oxidation by the CYP2C19 enzyme. Mutations in the CYP2C19 gene can thus influence both pharmacokinetics of PPI and as a result the suppressive effect on acid output.

In a Caucasian population, 60-70% of the population does not carry any mutation in the CYP2C19 gene (homozygous extensive metabolizers, *wt/wt* genotype), 30-40% has a point mutation in one allele of the CYP2C19 gene (heterozygous extensive metabolizers, *wt/*2* genotype), and 2-5% has a mutation in both alleles of the CYP2C19 gene (poor metabolizers, **2/*2* genotype) [7].

The influence of CYP2C19 genotype on pharmacokinetics and acid-inhibitory effect of omeprazole, the first PPI on the market, has been investigated in many studies during the last decades [8,9]. These studies showed that the metabolism of the *R*-isomer of the racemic mixture omeprazole was much more dependent on CYP2C19 than its S-isomer, esomeprazole. This implies that the pharmacokinetics and acid-inhibitory effects of esomeprazole are less affected by CYP2C19 polymorphisms than the pharmacokinetics and acid-inhibitory effects of omeprazole [10, 11]. The effect of CYP2C19 on the metabolism of pantoprazole is less well established. One study on the stereo-isomers of the racemic mixture pantoprazole in poor metabolizers of CYP2C19 showed that the metabolism of R(+)-pantoprazole is impaired to a greater extent than the metabolism of S(–)-pantoprazole [12]. These results indicated that the metabolism of pantoprazole also depends on CYP2C19.

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 PPI 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 [13]. 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 primary objective of this study therefore was to compare the acid-inhibitory effects of esomeprazole 40 mg and pantoprazole 40 mg at 4, 24 and 120 h after oral administration in a Caucasian population of *H. pylori*-negative subjects with known CYP2C19 genotype.

Secondary objectives were to describe the pharmacokinetics of esomeprazole 40 mg and pantoprazole 40 mg in relation to the pharmacodynamics and 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 August 2006. After inclusion, each subject was assigned to one of the two 5-day dosing periods with either oral esomeprazole 40 mg once daily (o.d.) or oral pantoprazole 40 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 randomized cross-over sequence, provided by a computer generated randomization list.

Sample Size

The power calculation is based on parametric assumptions. The primary outcome variable is percentage of time (during 24 hours) that pH is larger than 4. This variable is compared between two treatments (40 mg esomeprazole and 40 mg pantoprazole) in a 2-periods 2-treatment cross-over study. A clinically relevant mean difference of the outcome variable between the two treatments is 10 percent points. The standard deviation of the outcome variable is set at 16 percent points [6]. Assuming a Pearson correlation of 0.54 between the two measurements under consecutive treatments [4], the above clinically relevant mean difference is 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 pantoprazole, 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 glucose, serum creatinine, total bilirubin, serum alkaline phosphatase, serum ASAT and ALAT). They were eligible for inclusion if an *H. pylori* urea breath test (^{13}C Urea Breath Test, Simac Diagnostica, Veenendaal, the Netherlands) 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 [14]) 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 [6,15]. 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. After the dose, blood samples (5 mL) for determination of esomeprazole or pantoprazole serum concentrations were drawn at 30, 45, 60, 90 min, and at 2, 3, 4, 5, 6, 7, and 8 h. From 23:00 hours the subjects remained in fasting condition and slept. They arose again between 07:00 and 07:30 hours the next day. The pH electrode was removed at 08:55 hours (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). Before breakfast, the first blood sample (pre-dose) was drawn. Blood samples (5 mL) for determination of esomeprazole and pantoprazole serum concentrations were drawn at 1, 2, 3, 4, 5, 6, 7, and 8 h after intake of study medication. From 23:00 hours the subjects remained in fasting condition and slept. They arose again between 07:00 and 07:30 hours the next day. The pH electrode was removed at 08:55 hours (day 6) and the position of the assembly was checked prior to removal. Standard meals and drinks were provided as previously described [6, 15].

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.

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 (GastrograpH Mark II, SME Medizintechnik GmbH, Weil am Rhein, Germany). The sampling rate of these dataloggers is 4 per second. Every 2-s, the median of 8 voltage measurements was 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 s.

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 h after dosing, first 24 h (day 1) and last 24 h (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 [7]. This gain is represented as Δ percentage of time with intragastric pH > 4. A change in this Δ percentage 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 [16]. We defined individuals showing a Δ of ≥ 10% as responders and individuals with a Δ of < 10% as nonresponders.

Esomeprazole and pantoprazole assays

Serum concentrations of esomeprazole and pantoprazole were determined by means of liquid chromatography techniques (HPLC) at the laboratory of the Central Hospital Pharmacy, the Hague, the Netherlands [18]. The assays were linear in the range 0.025-2.5 mg/L for esomeprazole and 0.1-10 mg/L for pantoprazole. The inter-day precision of the assay ranged from 0.9-11.1% for esomeprazole and from 1.3-3.7% for pantoprazole; accuracy values were between -4.0 and +2.0% for esomeprazole and between +1.7 and +6.3% for pantoprazole.

Pharmacokinetic data

Pharmacokinetic models were fitted to data from all individuals simultaneously using non-linear mixed effects modelling (NONMEM (UCSF, San Francisco, USA), double precision; version V, level 1.1) [18]. The first-order conditional estimation method was used throughout the analysis taking into account interaction between inter-patient variability and residual variability. A one-compartment kinetic model with first-order absorption and first-order elimination was used to describe esomeprazole and pantoprazole plasma concentration-time curves. With the final population pharmacokinetic models available Bayesian analyses were performed to obtain individual values for area under the plasma concentration vs. time curve (AUC).

Statistical analysis

Statistical comparison between esomeprazole and pantoprazole administration was made by using a mixed model ANOVA with restricted maximum likelihood estimates for the effects. Complete and incomplete cases (n = 22, 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, eight parameters were estimated: six for the time effect (2 periods times, 3 days per period) and two for the treatment effect (esomeprazole – pantoprazole 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 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 pantoprazole. Statistical comparison between *wt/wt* and *wt/*2* under either treatment was made in complete and incomplete cases (n = 17, intention-to-treat). 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. The same model was used for statistical comparison between AUCs on day 1 and day 5 under either treatment (all complete cases, n = 19). Two-sided P-values < 0.05 were considered significant.

RESULTS

Twenty-two healthy subjects (9 male and 13 women, with a mean age of 22 (range 18-31) years, a mean body mass index of 21.8 (18.3 - 27.5) kg/m^2) were included in the study. Three subjects discontinued after the first treatment period: two subjects violated the study protocol and one subject emigrated unforeseen. Of the 19 subjects who completed the study, seven had *wt/wt* genotype, seven were *wt/*2*, two were *wt/*17*, two had **2/*17* genotype and one was **2/*2*. No **3* to **6* mutations were observed. Both drugs were well tolerated and there were no clinically relevant adverse events reported. Baseline intragastric pH data for both treatment periods for the total group of subjects are shown in Table 1a.

At day 1 and 5 of administration, esomeprazole treatment led to a significantly higher median intragastric pH and percentage of time with an intragastric pH > 4 than pantoprazole for the total group of subjects throughout the 24-h period as well as the upright period, but not during the supine period (Tables 1b and 1c and Figure 1).

Table 1A Mean (95% CI) of the % of time that the intragastric pH was < 4 (% pH < 4) at day 0 of both study periods in the total group of 22 subjects

Variable	Day 0	
	Period 1	*Period 2*
% pH < 4	87.7 (81.1 to 94.4)	88.65 (81.8 to 95.5)
% pH < 4 U*	91.0 (84.3 to 97.8)	92.5 (85.6 to 99.5)
% pH < 4 S*	81.4 (71.4 to 91.5)	81.3 (70.9 to 91.7)

**U: upright, S: supine*

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* = 22)

Variable	Day 1		Day 5	
	esomeprazole	*pantoprazole*	*esomeprazole*	*pantoprazole*
median pH	3.95 (3.5 - 4.3)	2.8 (2.4 - 3.2)	5.0 (4.6 - 5.4)	3.8 (3.4 - 4.2)
median pH U*	4.1 (3.8 - 4.5)	2.8 (2.4 - 3.1)	5.1 (4.75 - 5.5)	3.96 (3.6 - 4.3)
median pH S*	3.7 (3.0 - 4.4)	3.1 (2.5 - 3.8)	4.7 (4.0 - 5.4)	3.6 (2.9 - 4.3)
% pH > 4	51.9 (45.0 - 58.8)	32.9 (26.1 - 39.7)	72.6 (65.7 - 79.5)	49.4 (42.6 - 56.2)
% pH > 4 U	54.9 (47.9 - 61.8)	31.3 (24.5 - 38.2)	79.6 (72.6 - 86.6)	53.1 (46.2 - 60.0)
% pH > 4 S	46.4 (36.0 - 56.8)	36.1 (25.9 - 46.3)	60.3 (49.9 - 70.7)	42.5 (32.3 - 52.7)

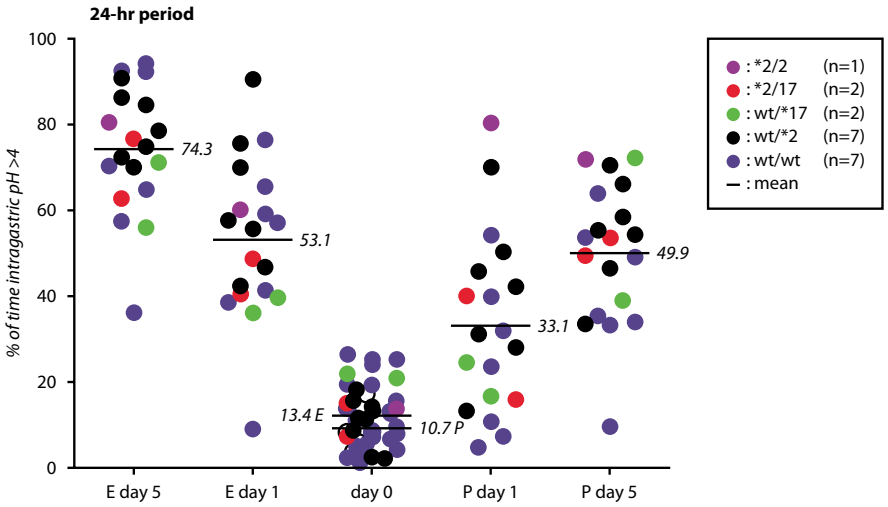
**U: upright, S: supine*

Table 1C Mixed model ANOVA estimates (95% CI) of the treatment effects (esomeprazole-pantoprazole) in the total group (*n* = 22)

	Day 1			Day 5		
Variable	effect	95 % CI	P	effect	95 % CI	P
median pH	1.14	0.7 - 1.5	0.000	1.2	0.8 - 1.6	0.000
median pH U*	1.35	1.0 - 1.7	0.000	1.2	0.8 - 1.5	0.000
median pH S*	0.56	-0.2 - 1.3	0.14	1.1	0.3 - 1.8	0.006
% pH > 4	19.0	11.2 - 26.8	0.000	23.2	15.4 - 31.0	0.000
% pH > 4 U	23.5	15.7 - 31.4	0.000	26.5	18.6 - 34.3	0.000
% pH > 4 S	10.3	-1.1 - 21.6	0.076	17.8	-6.5 - 29.1	0.002

**U: upright, S: supine*

Figure 1 Individual (*n* = 19) and mean values of percentage of time with intragastric pH > 4 during the 24-hr period at day 0, day 1 and day 5 of administration of esomeprazole 40 mg (E) and pantoprazole 40 mg (P)



With esomeprazole, 18 out of 19 subjects (95%) showed a response of ≥10% at day 1. With pantoprazole, 14 out of 19 subjects (74%) showed a response of ≥10%. 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%. During the first 4-h after the first dosing, esomeprazole provided higher median intragastric pH values and a larger increase in percentage of time with intragastric pH > 4 than pantoprazole (Figure 2). The median intragastric pH with esomeprazole was 2.55 (2.10-3.0) and with pantoprazole 1.94 (1.5-2.38) [treatment effect (esomeprazole-pantoprazole): 0.6, 95% CI: 0.08-1.13, $P = 0.010$]. The percentage of time with an intragastric pH > 4 of esomeprazole was 23.3% (15.2-31.4) and of pantoprazole 8.7% (0.8-16.6), (treatment effect: 14.6, 95% CI: -3.9-25.3, $P = 0.026$).

During pantoprazole administration, heterozygous carriage of a *CYP2C19*2* mutation resulted in significantly higher percentage of time with intragastric pH > 4 and median intragastric pH at day 1 (Figure 3) and at day 5 (% of time intragastric pH > 4: $P = 0.041$, median intragastric pH: $P = 0.043$). During administration of esomeprazole no significant differences between *wt/wt* and *wt/*2* genotypes were observed neither at day 1 (Figure 3) nor at day 5 (% of time intragastric pH > 4: $P = 0.568$, median intragastric pH: $P = 0.590$).

Figure 2 Median intragastric pH (over 10-minute time intervals) and 25 percentile in the first 4 hours after dosing of esomeprazole 40 mg (E) and pantoprazole 40 mg (P) at day 1 ($n = 19$)

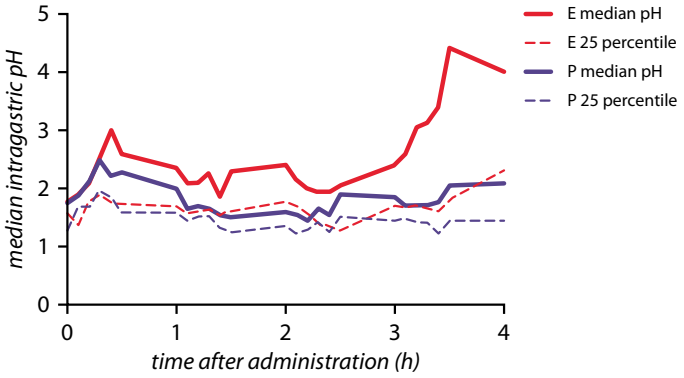
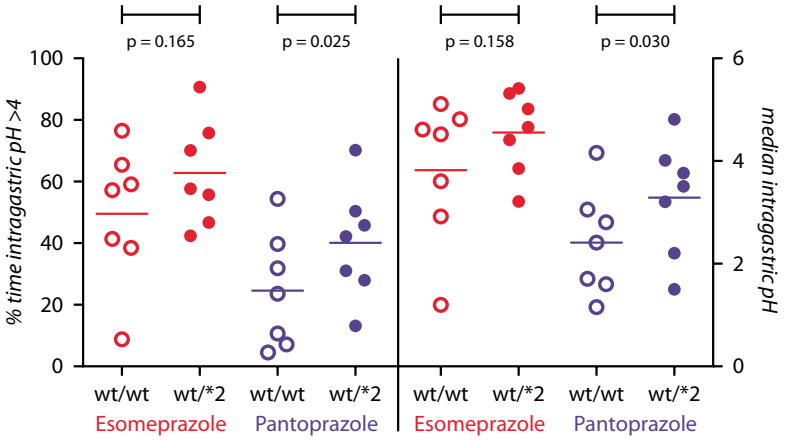


Figure 3 Individual and mean values of percentage of time with intragastric pH > 4 (left) and median intragastric pH (right) in *wt/wt* (closed dots, $n = 7$) and *wt/*2* (open dots, $n = 7$) subjects after administration of esomeprazole 40 mg or pantoprazole 40 mg at day 1



Pharmacokinetic analysis: the 19 subjects who completed the study, showed during esomeprazole treatment a significant increase in AUC from day 1 to day 5. However, there was no difference in AUC between *wt/wt* and *wt/*2* subjects at day 1 and day 5. During pantoprazole treatment, there was no increase in AUC from day 1 to day 5, but at day 1 and day 5 AUC was significantly higher in *wt/*2* subjects than in *wt/wt* subjects (Table 2, Figure 4).

Table 2 Mixed model ANOVA estimates (95% CI) of the mean AUC of esomeprazole and pantoprazole of the total group and differentiated to genotypes at day 1 and day 5

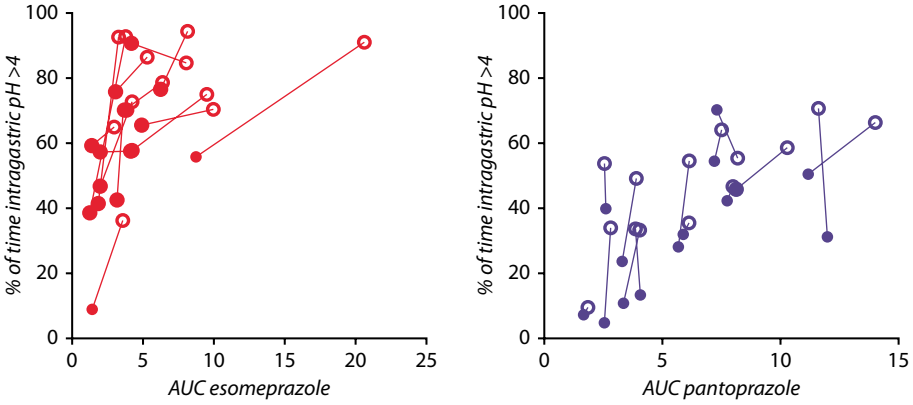
Variable		esomeprazole 40 mg		pantoprazole 40 mg	
AUC	*n*	day 1	day 5	day 1	day 5
total group	19	3.16 (0.34 - 6.0)	6.35 (3.52 - 9.18)$^{\#}$	6.79 (3.96 - 9.61)	8.14 (5.31 - 10.98)$^{\wedge}$
wt/wt	7	2.68 (0.20 - 5.17)*	5.10 (2.62 - 7.59)$^{\&}$	3.82 (1.34 - 6.31)$^{@}$	4.12 (1.64 - 6.60)$^{\$}$
*wt/*2*	7	4.24 (1.75 - 6.72)	8.22 (5.74 - 10.70)	7.95 (5.47 - 10.43)	8.90 (6.42 - 11.38)

P-value: day 1 vs. day 5: esomeprazole: $^{\#}$0.034, pantoprazole: $^{\wedge}$0.357

*P-value: wt/wt vs. wt/*2: esomeprazole day 1: *0.369, esomeprazole day 5: $^{\&}$0.079*

*P-value: wt/wt vs. wt/*2: pantoprazole day 1: $^{@}$0.023, pantoprazole day 5: $^{\$}$0.010*

Figure 4 Relationship between percentage of time with intragastric pH > 4 and AUC in *wt/wt* and *wt/*2* subjects (*n* = 14) after administration of esomeprazole and pantoprazole at day 1 (closed dots) and day 5 (open dots)



DISCUSSION

The aim of the study was a comparison of the acid-inhibitory effects of esomeprazole and pantoprazole in relation to CYP2C19 polymorphism and not a comparison of the therapeutic effects of both PPIs. Therefore, the intragastric pH studies were carried out in a population of healthy *H. pylori*-negative subjects. In crossover-studies, intra-individual comparisons may be affected by the *H. pylori* status of the subjects. Treatment with antisecretory agents may alter the pattern of *H. pylori* infection, thus introducing a carry-over effect in cross-over studies with subsets of *H. pylori*-positive subjects [15, 19, 20].
The results of this direct comparative study in *H. pylori*-negative subjects showed a significantly better acid-suppressive effect of single as well as repeated administration of esomeprazole 40 mg compared to pantoprazole 40 mg. When looking at both 4-h and 24-h and 120-h post-dosing periods, the median 24 hour intragastric pH and the percentage of time with a pH above 4 were significantly higher with esomeprazole.

These findings are consistent with previous studies that compared esomeprazole 40 mg with pantoprazole 40 mg. In one study in *H. pylori*-negative patients with symptoms of GERD, esomeprazole on day 1 maintained an intragastric pH > 4 during 50% of time and pantoprazole during 29% of time. This difference remained present at day 5 (67 vs. 45%) [21]. However, in this study the acid-inhibitory effects of both PPIs were not investigated in relation to baseline pH, pharmacokinetics and genotype. In another study, *H. pylori*-negative patients with GERD were analysed in a comparative cross-over design with 5 PPIs. On day 5, intragastric pH was > 4.0 during 58% of time with esomeprazole and during 42% of time with pantoprazole [22]. This study did not investigate the acid-inhibitory effects after single administration and did not examine acid inhibition in relation to baseline pH, pharmacokinetics and pharmacogenetics. This implies that only the effect during steady state was studied. By studying baseline intragastric pH, data can be translated into the proportion of patients that has any acid-inhibitory effect on a single dose of a specific PPI; an effect which we defined in our study as at least 10% reduction of the percentage of time with pH > 4 compared to baseline. In this study as well as in a previous study from our group [7], we found that 26-31% of patients have no response after a single dose of pantoprazole, vs. 5% of the same patients not showing any response after esomeprazole.

Although PPIs were devised for continuous therapy only, they are in clinical practice mostly used on intermittent basis [13], on demand or short-term treatment. On-demand and short-term treatment require a fast and reliable onset of drug action. In this respect, the higher proportion of responders with esomeprazole compared with pantoprazole is clinically relevant. Besides a significantly higher median intragastric pH and percentage of time with pH > 4 with esomeprazole, our data show that esomeprazole 40 mg provided a faster onset (defined as the timepoint where intragastric pH reaches 4, see Figure 2) than pantoprazole 40 mg. With esomeprazole, an intragastric pH of 4 was reached 3.5 h after administration. With pantoprazole a pH of 4 was reached 5.5 h after administration (data not shown for pantoprazole). Data from intravenous administration of esomeprazole 40 mg and pantoprazole 40 mg confirm the faster mode of action of esomeprazole during the first 4-h of administration [23].

The pharmacokinetic data of pantoprazole from this study are in accordance with previous data [6]. For pantoprazole, the AUC following repeated administration was similar to the AUC after a single dose, indicating that the bioavailability and oral clearance remained constant over time. The AUC of esomeprazole was significantly higher on day 5 than on day 1 with a 2-fold increase. This effect has been described before with other studies reporting a 2.4–2.6-fold increase from day 1 to 5 of treatment [10,24,25]. The increased AUC of esomeprazole at day 5 results from a decreased metabolic rate, which has been shown to be a combination of decreased first-pass elimination and decreased systemic clearance [10]. A likely explanation for these effects is auto-inhibition of the major esomeprazole metabolizing enzyme CYP2C19. This can be caused either by esomeprazole itself or by the sulphone metabolite, which has been demonstrated, to inhibit CYP2C19 hydroxylation and demethylation steps [26]. Although there is influence of CYP2C19 on esomeprazole clearance, its metabolic pathway is less influenced by CYP2C19 than is omeprazole [10]. In more detail, in vitro data demonstrated that with esomeprazole more of the sulphone metabolite is formed (CYP3A4 dependent) and less of the hydroxyl metabolite (CYP2C19 dependent), indicating that the dependence on CYP2C19 relative to CYP3A4 is less for the metabolism of esomeprazole than that of omeprazole [10,27]. Our data show that auto-inhibition of CYP2C19 by esomeprazole occurs at the same level in both *wt/wt* and *wt/*2* genotypes, as both genotypes showed a comparable increase in AUC from day 1 to day 5.

Our study has demonstrated that although a significant auto-inhibition of metabolism occurs, the pharmacokinetics and pharmacodynamics of esomeprazole are not influenced by *CYP2C19* genotype in *wt/wt* and *wt/*2* subjects. In contrast, both the pharmacokinetics and pharmacodynamics of pantoprazole were influenced by CYP2C19 genotype, with a lower AUC and less acid-inhibition in *wt/wt* subjects compared to *wt/*2* subjects.

Pharmacokinetic data have shown that pantoprazole metabolism is stereoselective and dependent on CYP2C19 status in extensive and poor metabolizers [12,28]. Pharmacodynamic data of pantoprazole related to CYP2C19 polymorphism in Caucasians are lacking. We have previously shown that subjects with a *wt/*17* mutation, a mutation associated with an increased CYP2C19 metabolic capacity [29],did not show significant acid-inhibition after a single dose of pantoprazole [7]. However, that study was designed to investigate the intra-individual influence of CYP2C19 mutations on the acid-inhibitory effect (comparison with a subjects' intragastric pH at baseline), rather than to study the inter-individual effect between subjects with *wt/wt* and *wt/*2* genotype. Overall, data from this study and from our previous study indicate that pantoprazole shows genotype dependent acid-inhibition. This results in less acid-inhibition in subjects with *wt/*17* genotype, unaffected acid-inhibition in subjects with *wt/wt* genotype and stronger acid-inhibition in subjects with *wt/*2* and *2/*2* genotype.

The PK-PD studies have demonstrated that the AUC of omeprazole and esomeprazole show a good correlation with the percentage of time with pH > 4 [30,31]. The PK-PD data from the present study confirm that with esomeprazole an increase in AUC results in an increase in the percentage of time with pH > 4. With pantoprazole, also a PK-PD correlation was observed; however, the maximum effect was markedly lower than with esomeprazole. This observation raises the question whether pantoprazole shows a maximum acid-inhibitory effect after administration of 40 mg. Data from other studies not only demonstrate that pantoprazole shows a linear dose-effect relationship in the range of 10-40 mg once daily [32] but also show that increasing the dose above 40 mg does not lead to an increased median pH elevation [33-35]. The acid-inhibitory effect of esomeprazole increases after splitting the dose into 20 mg twice daily. However, pantoprazole 20 mg twice daily is as effective as 40 mg once daily [36]. Increasing the pantoprazole dose to 40 mg twice daily led to an acid-inhibitory effect with a percentage of time with pH > 4 of 70.8% at day 5 [37]. These pharmacodynamic data support the hypothesis that pantoprazole reaches a maximum acid-inhibitory effect at about 70%.

In Caucasian populations, 60-70% of the subjects have the homozygous genotype (*wt/wt*, homEM) for CYP2C19 and only 30-40% have a **2* mutation in one allele (*wt/*2*, heterozygous genotype, hetEM). In Asian populations, 50% of the subjects have a *wt/*2* or *wt/*3* genotype and 25% have mutations in both alleles (**2/*2* or **3/*3* genotype, poor metabolizer, PM). The question arises whether the doses chosen for *H. pylori*-eradication therapy or treatment of erosive reflux oesophagitis in Asian populations is effective in Caucasian populations. Clinical studies have shown that PMs and hetEMs benefit from an approximately 18% higher *H. pylori*-eradication rate compared to homEMs when standard dosages of PPIs are administered orally [38,39]. In a study with lansoprazole, healing rates at 4 weeks of erosive reflux oesophagitis were 15% higher in PMs than in homEMs and at 8 weeks 22% [40]. This calls either for higher dosages for all Caucasian patients or for genotype-based dosing. The therapeutic effects of pantoprazole and esomeprazole as a function of CYP2C19 genotype therefore need to be re-evaluated in an appropriate study design in patients with upper gastrointestinal disorders with and without chronic *H. pylori* infection.

CONCLUSION

Once-daily dosing with esomeprazole 40 mg orally provides a more effective and faster acid-inhibitory effect than pantoprazole 40 mg orally. Esomeprazole shows a higher rate of responders after single and multiple dosing than pantoprazole. In contrast to esomeprazole, pantoprazole metabolism is influenced by CYP2C19 polymorphism. In the Caucasian population control of intragastric acidity with pantoprazole is more unpredictable than control with esomeprazole.

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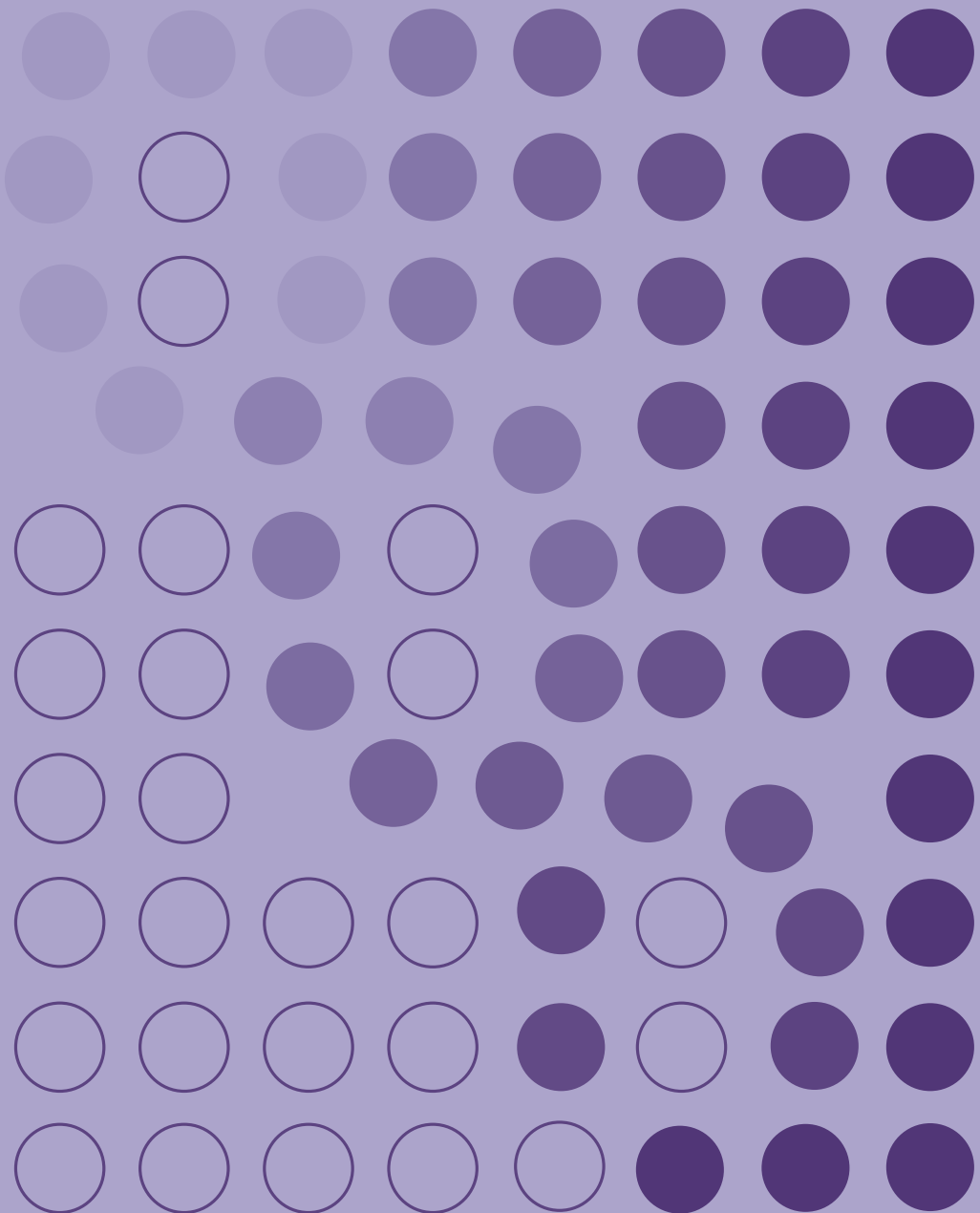
Chapter 6



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

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

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 (^{13}C Urea Breath Test, Simac Diagnostica, Veenendaal, the Netherlands) 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

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/m^2) 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.

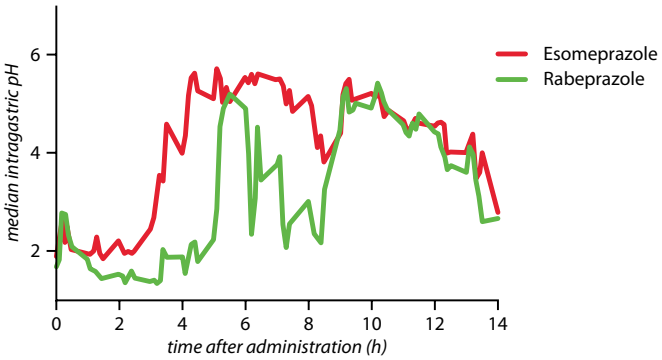
Table 1A Mean (95% CI) of the % of time that the intragastric pH was > 4 at day 0 of both study periods in the total group of 18 subjects

Variable	Day 0	
	Period 1	*Period 2*
% pH > 4 24h	12.4 (8.3 to 16.5)	9.5 (5.6 to 13.3)
% pH > 4 U*	8.4 (6.0 to 10.8)	8.2 (4.3 to 12.0)
% pH > 4 S*	20.1 (10.4 to 29.8)	12.0 (3.3 to 20.7)

**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)

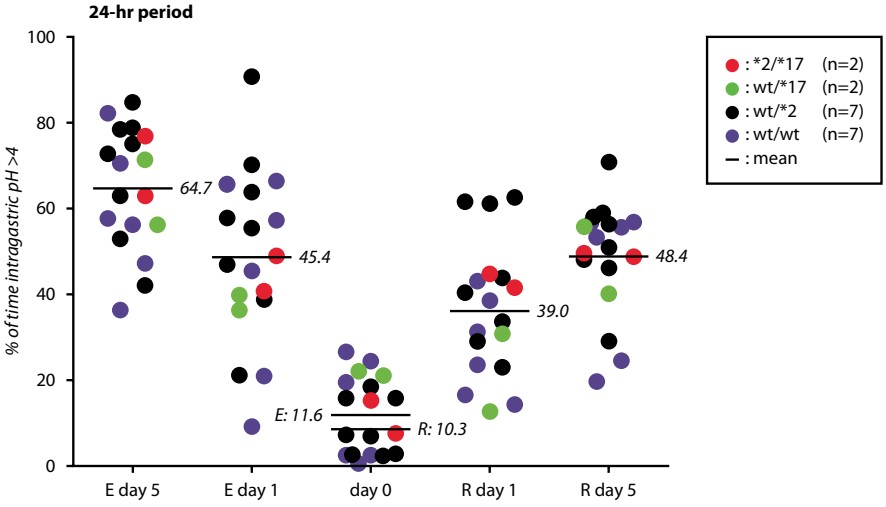


Table 1B Mixed model ANOVA estimates (95% CI) of the mean levels of median pH and % pH > 4 during treatment, adjusted for a possibly confounding time effect, in the total group (*n*=18)

Variable	Day 1		Day 5	
	esomeprazole	*rabeprazole*	*esomeprazole*	*rabeprazole*
median pH 24h	3.7 (3.1 - 4.2)	3.0 (2.4 - 3.5)	4.7 (4.3 - 5.1)	3.8 (3.4 - 4.3)
median pH U*	3.8 (3.2 - 4.4)	3.3 (2.7 - 3.9)	5.2 (4.8 - 5.5)	3.9 (3.6 - 4.3)
median pH S*	3.0 (2.4 - 3.7)	3.2 (2.5 - 3.8)	3.7 (2.8 - 4.5)	3.5 (2.7 - 4.3)
% pH > 4	45.4 (36.8 - 54.0)	39.0 (30.4 - 47.6)	64.6 (57.8 - 71.5)	48.4 (41.6 - 55.2)
% pH > 4 U	52.2 (42.5 - 62.0)	40.3 (30.7 - 50.0)	76.2 (68.5 - 84.0)	52.9 (45.1 - 60.6)
% pH > 4 S	33.1 (21.0 - 45.2)	36.4 (24.3 - 48.5)	41.4 (30.0 - 52.7)	41.7 (30.3 - 53.0)

**U: upright, S: supine*

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

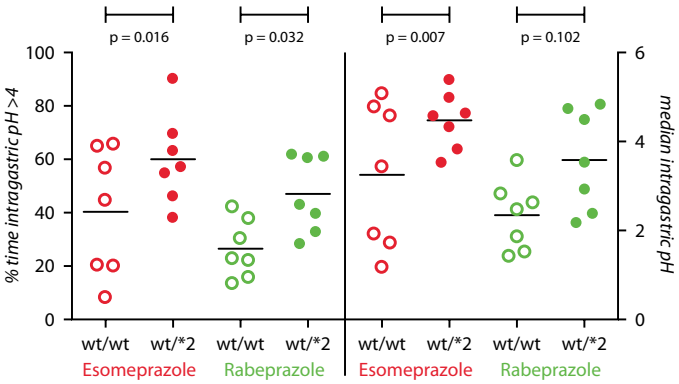
	Day 1			Day 5		
Variable	effect	95 % CI	P	effect	95 % CI	P
median pH 24h	0.68	0.2 - 1.1	0.008	0.86	0.61 - 1.1	0.000
median pH U*	0.50	-0.03 - 1.1	0.062	1.23	0.9 - 1.6	0.000
median pH S*	-0.16	-0.6 - 0.3	0.440	0.15	-0.7 - 1.0	0.724
% pH > 4	6.4	-0.1 - 12.9	0.054	16.3	10.9 - 21.6	0.000
% pH > 4 U	11.9	4.7 - 19.1	0.003	23.4	16.7 - 30.0	0.000
% pH > 4 S	-3.3	-14.0 - 7.4	0.518	-0.33	-11.9 - 11.2	0.952

**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:

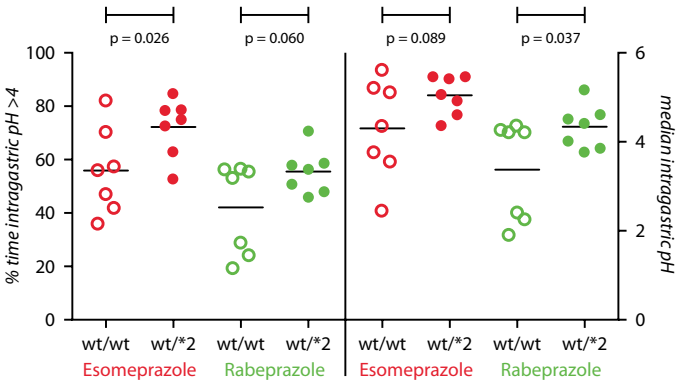


Table 2A Mixed model ANOVA estimates (95% CI) of the mean levels of median pH and treatment effect (*wt/wt – wt/*2*) on day 1 and 5 of administration of esomeprazole (*n*=18)

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

**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)

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

**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 *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 *wt/wt* and *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 *wt/wt* and *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 *Ki* [44]. The *Ki* 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 *Ki*. During clearance of the drug, this effect will reverse: the *Ki* 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 *wt/wt* and *wt/*2* genotypes with a twice daily dosing schedule of esomeprazole and rabeprazole.

For esomeprazole, the results between *wt/wt* and *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 *wt/wt* and *wt/*2* genotypes. Although the studies were identical in design and powered to detect significant differences between *wt/wt* and *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.

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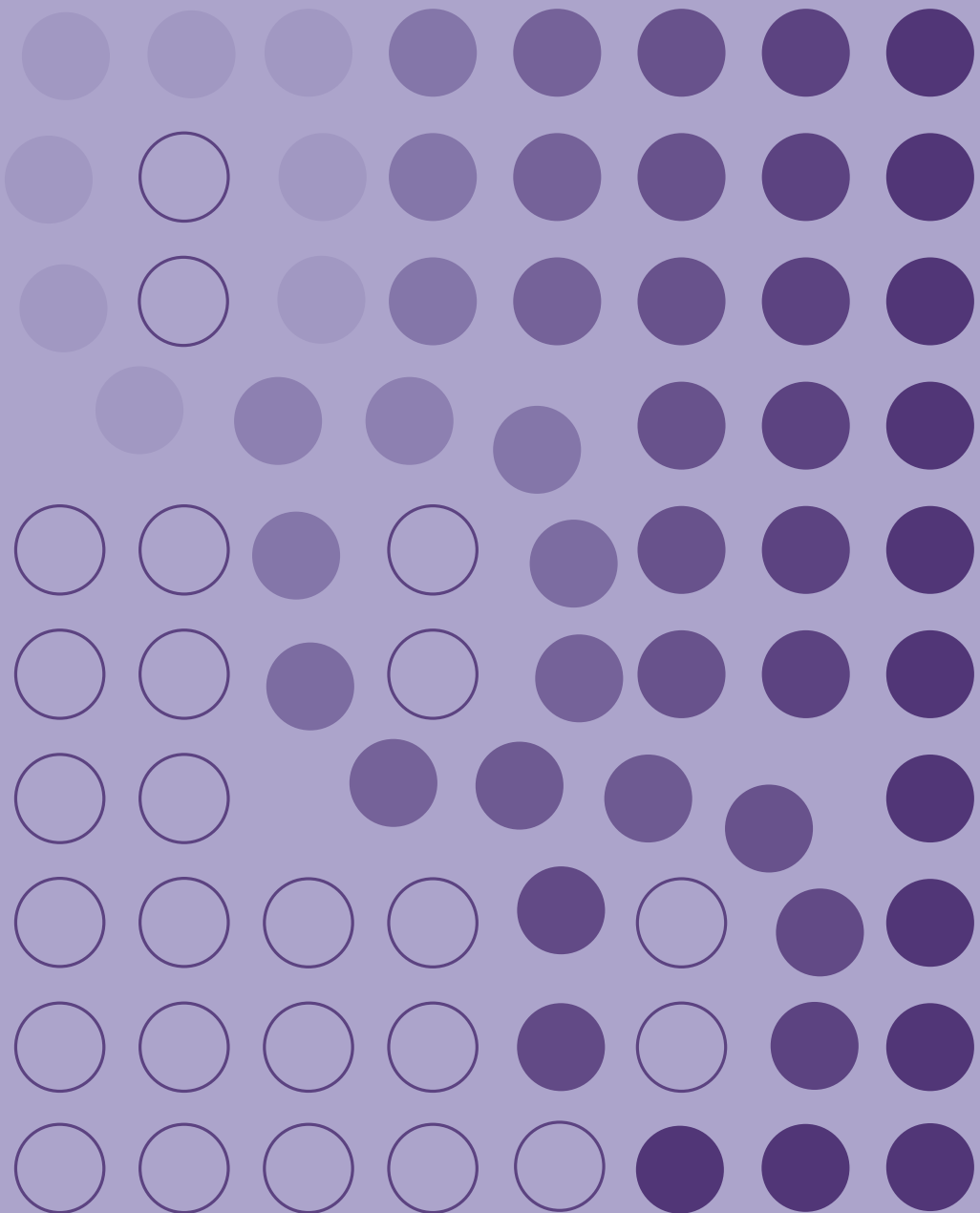
Chapter 7



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

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7 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%).

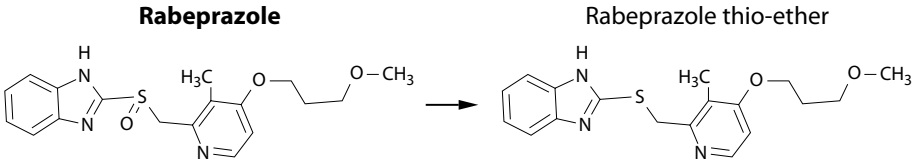
Conclusion

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.

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

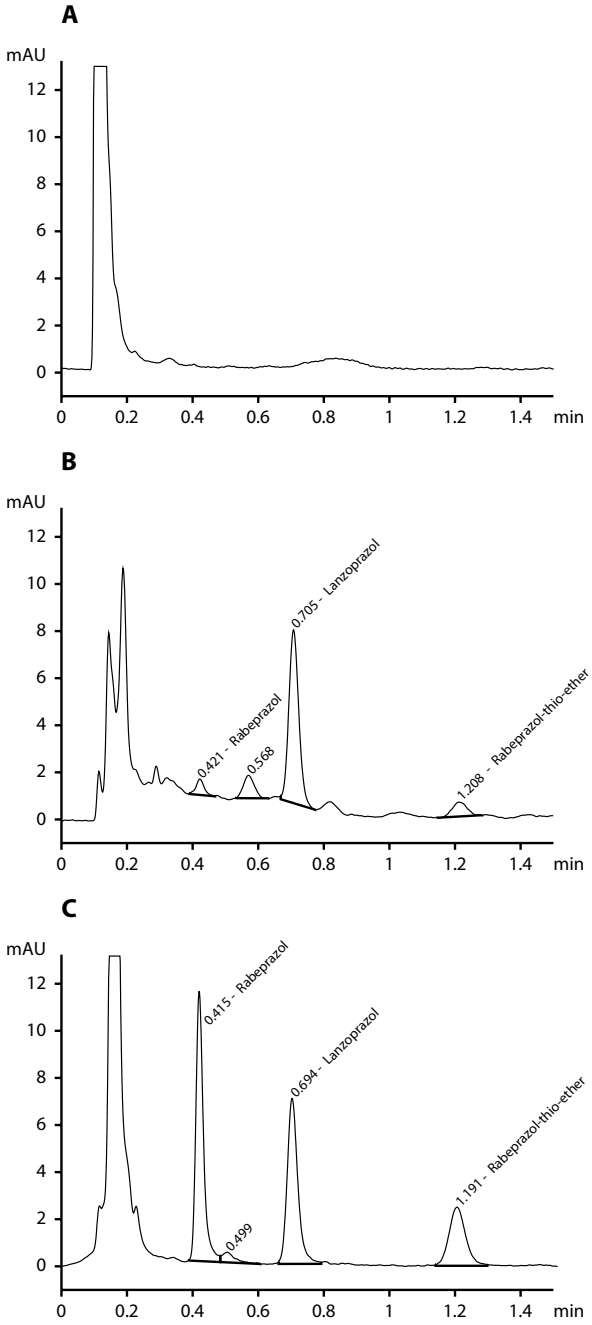
Chromatographic conditions

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, CV = 11.9%), and for rabeprazole thio-ether 0.026 mg/L (n = 6, 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)

		Intra-day			Inter-day			Recovery (%)	
Compound	Added (mg/L)	Found mean	CV (%)	Bias (%)	Found mean	CV (%)	Bias (%)	Found mean	CV (%)
Rabeprazole	0.015	0.0169	2.9	11.6	0.0161	11.9	6.6		
	0.252	0.2553	2.2	1.2	0.2621	3.1	3.9	75.7	2.6
	0.706	0.7326	1.7	3.7	0.7346	2.0	4.0		
Rabeprazole	0.026	0.0281	8.1	8.0	0.0262	12.6	0.7		
thio-ether	0.521	0.5398	1.6	3.7	0.5381	1.0	3.4	99.9	1.3
	1.041	1.0753	1.1	3.3	1.0775	1.0	3.5		

Stability during processing and storage

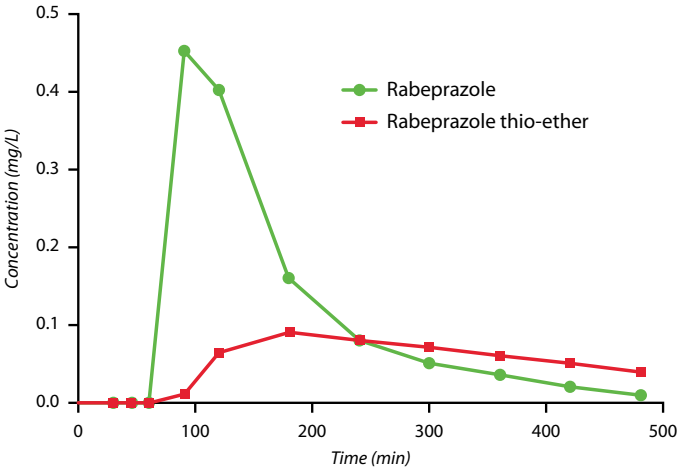
Rabeprazole and its metabolites were shown to be unstable in serum samples without taking precautions. Samples stored at room temperature and at –70°C showed a rapid decomposition for rabeprazole and its metabolites (data not shown). For this reason, samples had to be stabilised using DEA, as has been 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_{max} of 0.26 mg/L, a T_{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_{max} of 0.069 mg/L, a T_{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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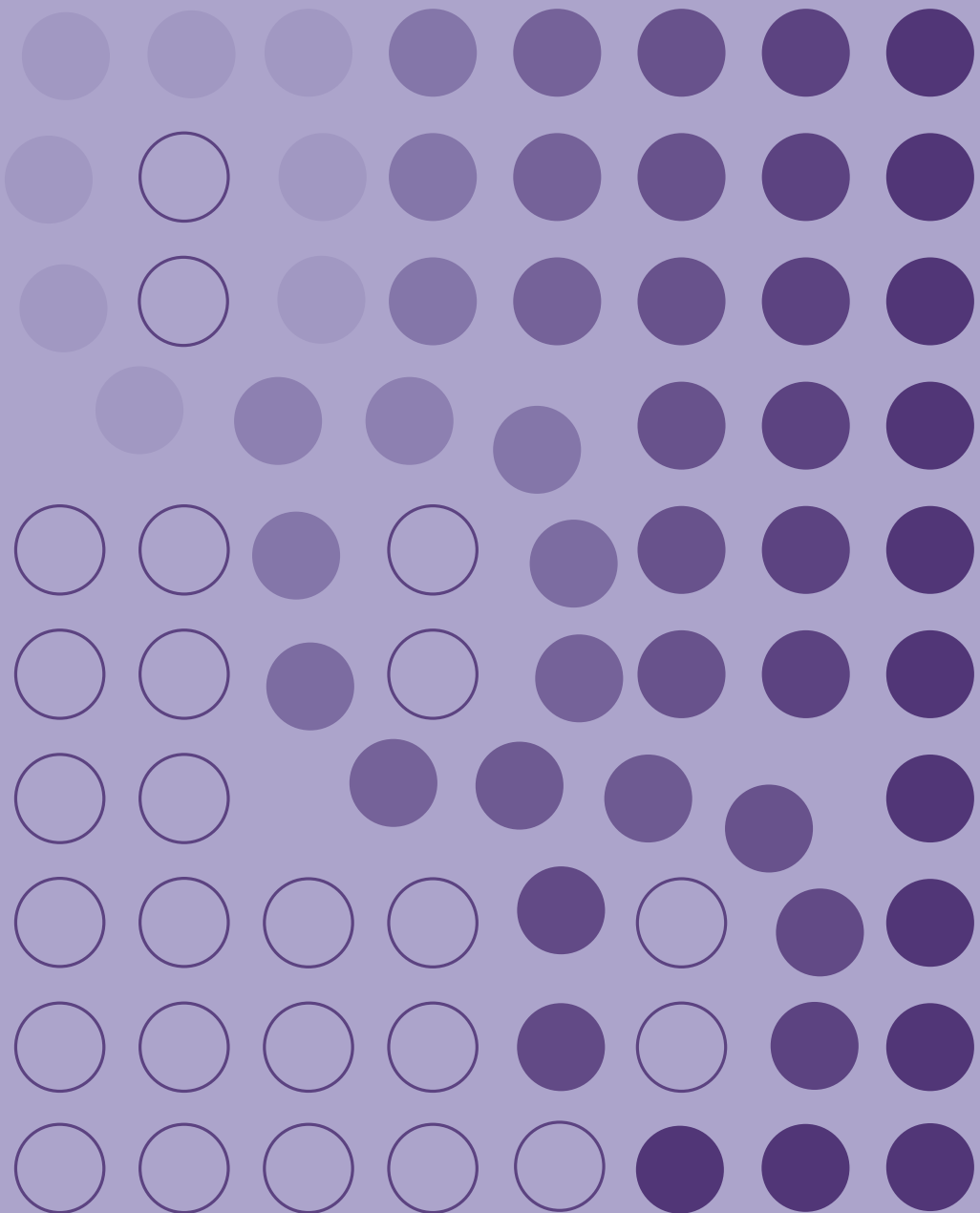
Chapter 8



Systematic review: the influence of CYP2C19 polymorphism on the acid-inhibitory effects of proton pump inhibitors

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Submitted

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8 Systematic review: the influence of CYP2C19 polymorphism on the acid-inhibitory effects of proton pump inhibitors

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]:

Table 1 Level of evidence and definitions

Level of evidence	Definition
0	data on file
1	incomplete, published case reports or abstracts
2	well-documented, published case reports; retrospective analyses of case series
3	controlled, published pharmacogenetic studies of moderate quality in patients or healthy volunteers with endpoints (moderate is defined as limited patient numbers, impaired strength of the study, not randomized, not blinded, cohort studies or case-control studies)
4	controlled, published pharmacogenetic studies of good quality (e.g. randomized, blinded) in patients or healthy volunteers with endpoints

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.

Table 2 Magnitude of clinical outcome and definitions

Score	Definition
A	no or minor effect: no significant difference between genotypes
B	moderate effect: defined as a significant difference in 24-hour mean or median intragastric pH between genotypes, or a significant difference in proportion of time with 24-hour intragastric pH above pH threshold 4, without clinical consequences
C	major effect: defined as a significant difference between genotypes in proportion of time with intragastric pH above pH threshold 4, leading to a response (defined as an intragastric pH above 4 for more than 83.3% of the time) in one of the phenotypic subgroups compared with other phenotypic subgroups with no response (defined as an intragastric pH above 4 equally or less than 83.3% of time above pH 4 [47, 48].

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

A: esomeprazole

ref	daily dose	end point	day	study design	race	*n*	*n*			outcome clinical endpoint			level of evidence and magnitude		
							homEM	hetEM	PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM
[32]	40 mg	pH > 4	1 5	RD, CO, IB	Cau	15	7	7	1	NS NS	-	-	4A 4A	-	-
[33]	40 mg	pH > 4	1 5	RD, CO, IB	Cau	15	7	7	1	P=0.016 P=0.026	-	-	1B 1B	-	-
[32]	40 mg	pH IG	1 5	RD, CO, IB	Cau	15	7	7	1	NS NS	-	-	4A 4A	-	-
[33]	40 mg	pH IG	1 5	RD, CO, IB	Cau	15	7	7	1	P=0.007 NS	-	-	1B 1A	-	-

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.

B: lansoprazole

ref	daily dose	end point	day	study design	race	*n*	*n*			outcome clinical endpoint			level of evidence and magnitude		
							homEM	hetEM	PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM
[34]	2x 30 mg	pH > 4	7	RD, PC, DB, CO	J	20	6	9	5	-	P=0.04	P=0.04	-	4C	4C
[35]	30 mg	pH > 4 N	8	RD, PC, DB, CO	J	18	7	7	4	P=0.04	P=0.01	NS	4B	4B	4A
[29]	30 mg	pH > 4 D	7	Open, R	J	20	7	9	4	-	P<0.01	P<0.05	-	3C	3C
[37]	30 mg	pH IG	8	RD, PC, DB, CO	J	15	7	5	3	NS	P=0.02	NS	4A	4B	4A

C: omeprazole

ref	daily dose	end point	day	study design	race	*n*	*n*			outcome clinical endpoint			level of evidence and magnitude		
							homEM	hetEM	PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM
[31]	10 mg 20 mg	pH > 4	7	RD, CO, PG	J	18	6	6	6	- -	P<0.01 P<0.01	P<0.01 P<0.01	- -	3B 3C	3B 3C
[28]	20 mg	pH > 4	1 8	Open	C	18	6	6	6	P=0.038 NS	P<0.001 P=0.006	P=0.013 P=0.014	3B 3A	3B 3B	3B 3B
[21]	20 mg	pH IG	1	RD, PC, DB, CO	J	15	5	4	6	P=0.04	P=0.0001	P=0.03	4B	4B	4B
[36]	20 mg	pH IG	1 8	RD, PC, DB, CO	J	15	6	5	4	P=0.0096 P=0.0017	p=0.0098 P=0.010	P=0.0036 NS	4B 4B	4B 4B	4B 4A
[28]	20 mg	pH IG	1 8	Open	C	18	6	6	6	P=0.018 NS	P<0.001 P=0.0011	P=0.016 P=0.031	3B 3A	3B 3B	3B 3B

D: pantoprazole

ref	daily dose	end point	day	study design	race	*n*	*n*			outcome clinical endpoint			level of evidence and magnitude		
							homEM	hetEM	PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM
[32]	40 mg	pH > 4	1 5	RD, CO, IB	Cau	15	7	7	1	P=0.025 P=0.041	-	-	4B 4B	-	-
[32]	40 mg	pH IG	1 5	RD, CO, IB	Cau	15	7	7	1	P=0.030 P=0.043	-	-	4B 4B	-	-

E: rabeprazole

ref	daily dose	end point	day	study design	race	*n*	*n*			outcome clinical endpoint			level of evidence and magnitude		
							homEM	hetEM	PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM	homEM vs. hetEM	homEM vs. PM	hetEM vs. PM
[29]	20 mg	pH > 4	7	Open, R	J	20	7	9	4	-	NS	NS	-	3A	3A
[26]	20 mg 40 mg	pH > 4	8	RD, PC, DB, CO	J	15	5	6	4	NS NS	P<0.05 P<0.05	P<0.05 P<0.05	4A 4A	4C 4C	4C 4C
[30]	10 mg 20 mg 2x 10 mg	pH > 4	7	Open, RD, CO	J	18	6	6	6	NS NS NS	NS p<0.05 NS	NS NS NS	4A 4A 4A	4A 4C 4A	4A 4A 4A
[25]	10 mg 20 mg	pH > 3	1	RD, PC, PG	J	15	5	6	4	NS NS	NS NS	NS NS	3A 3A	3A 3A	3A 3A
[33]	20 mg	pH > 4	1 5	RD, CO, IB	Cau	15	7	7	1	P=0.032 P=NS	- -	- -	1B 1A	- -	- -
[36]	20 mg	pH IG	1 8	RD, PC, DB, CO	J	15	6	5	4	NS NS	P=0.010 NS	NS NS	4A 4A	4B 4A	4A 4A
[27]	20 mg	pH IG	1 8	Open	C	20	7	6	7	NS	NS	NS	3A 3A	3A 3A	3A 3A
[25]	10 mg 20 mg	pH IG	1	RD, PC, PG	J	15	5	6	4	NS NS	P=0.03 P=0.03	NS NS	3A 3A	3B 3B	3A 3A
[33]	20 mg	pH IG	1 5	RD, CO, IB	Cau	15	7	7	1	NS P=0.037	-	-	1A 1B	- -	- -

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 PPIs for Caucasian subjects or to switch to a less CYP2C19-dependent PPI needs further research, especially in homEMs and RMs.

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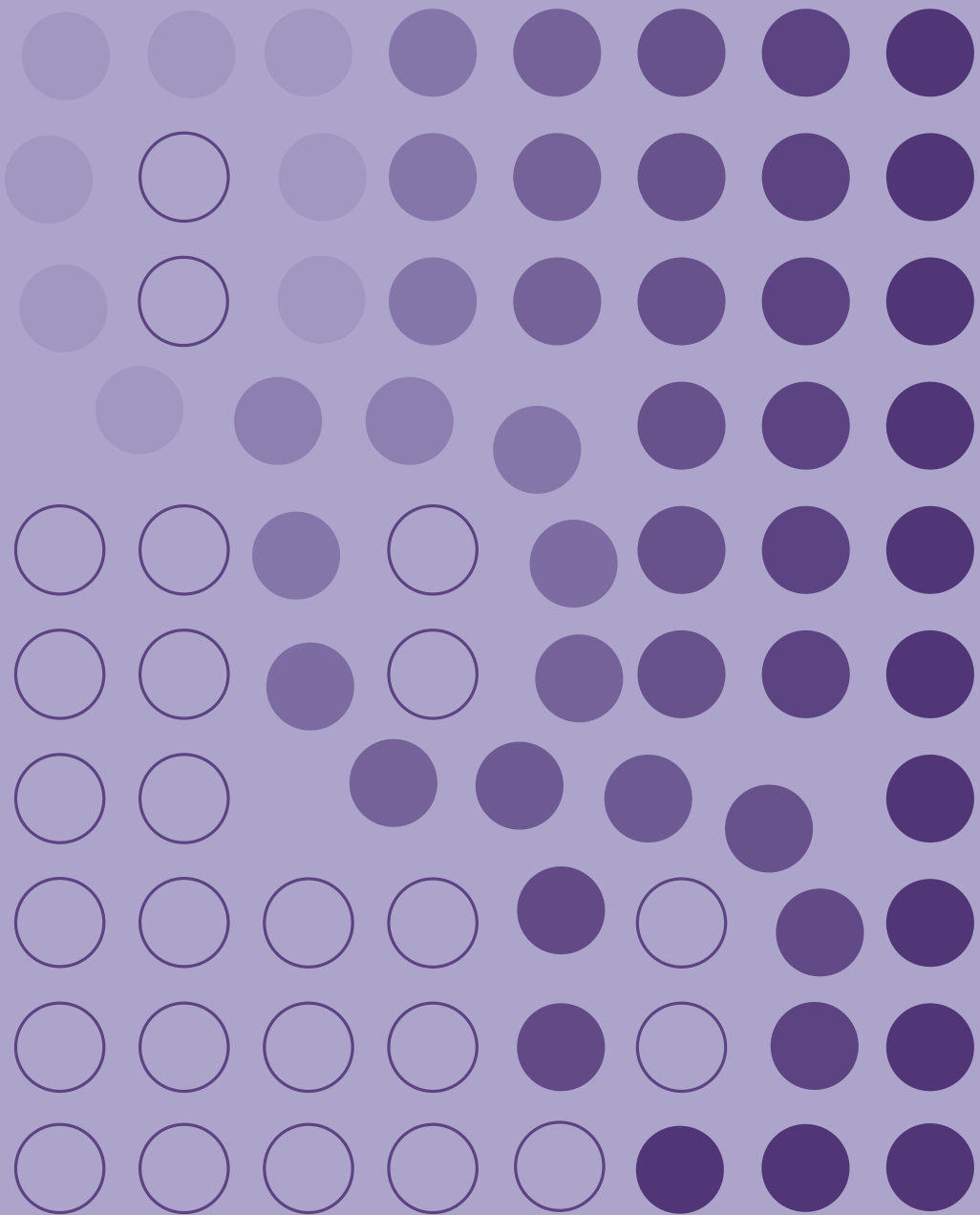
Chapter 9



General discussion

9

9 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[†]

CYP2C19 variant	Possible genotypes	Genotype prevalence in:			Effects:		Phenotype
		Caucasians (Dutch) (%)	Asians (Chinese) (%)	Asians (Japanese) (%)	kinetics: AUC[$]	dynamics: acid-inhibition	
**17*	**17/*17*	1.5	0	0	decreased	decreased	(U)RM
	**1/*17*	25	1.3	1.1			RM
	**2/*17*	8	0	1.5			RM?^
	**3/*17*	0	0	[*]			RM?^
**1*	**1/*1*	39	52.1	35.5	normal	normal	homEM
**2*	**1/*2*	25	33.7	40.2	increased	increased	hetEM
	**2/*2*	2 .9 (1.5)	9.2	9.7			PM
**3*	**1/*3*	0.2 (< 0.3)	6.1	14.7	increased	increased	hetEM
	**2/*3*	0	2.8	7.4			PM
	**3/*3*	0	0	2.4			PM

[†]: Since data about the prevalence of genotypes from different references are combined, the total percentage of 100% can be exceeded.
[$]: area under the concentration time curve
*[*]: 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/*1* 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 lansoprazole15 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_{max}), clearance (CL) and the AUC.
PPIs have shown a poor correlation between the C_{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_{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].

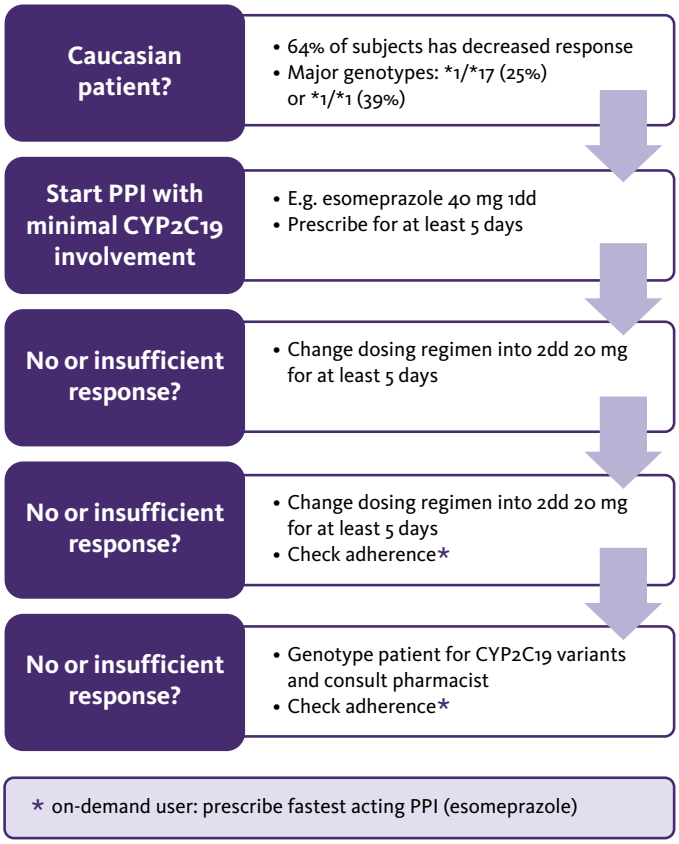
REBOUND ACID HYPERSECRETION (RAHS)

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]



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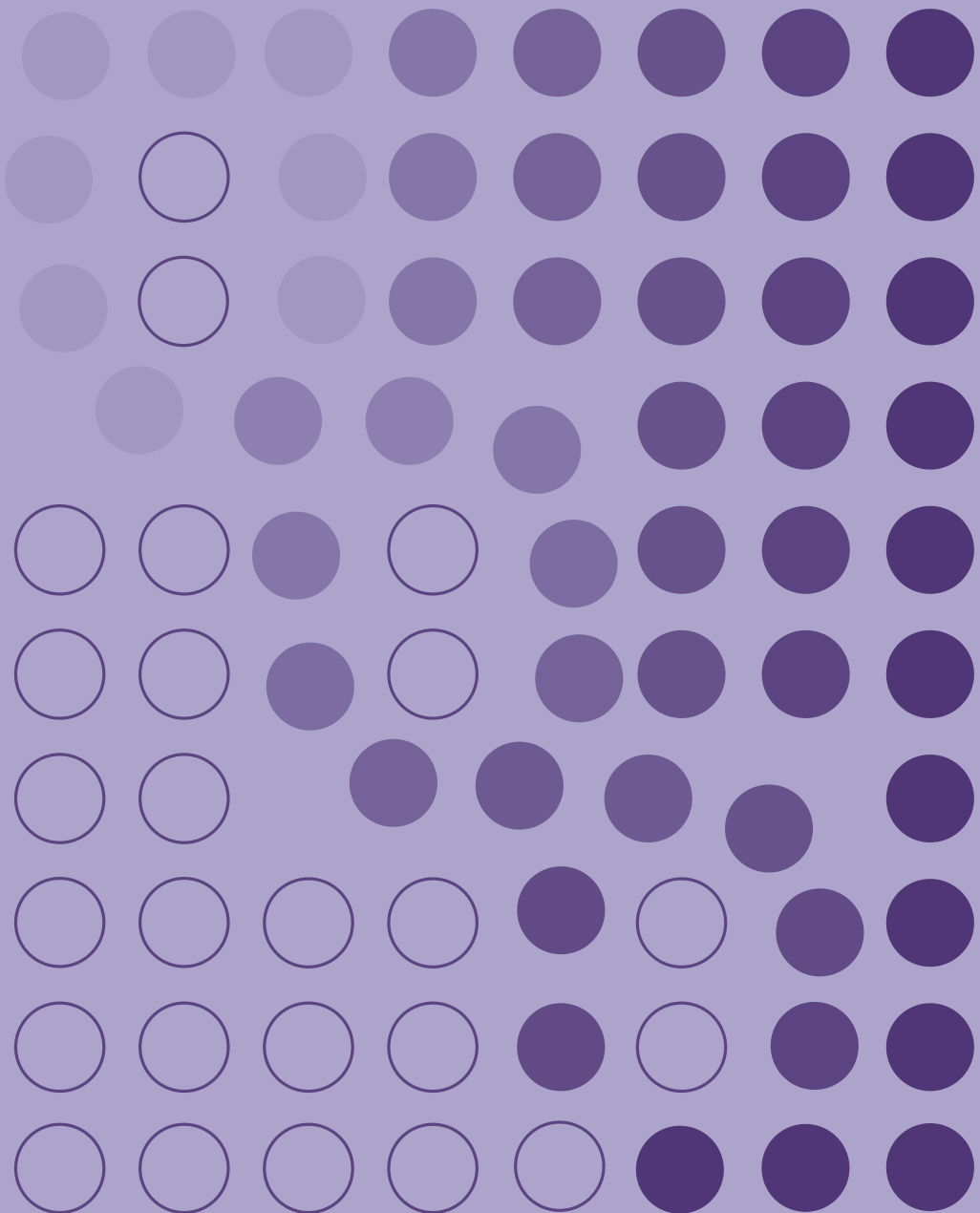
Chapter 10



Summary

10

10 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.

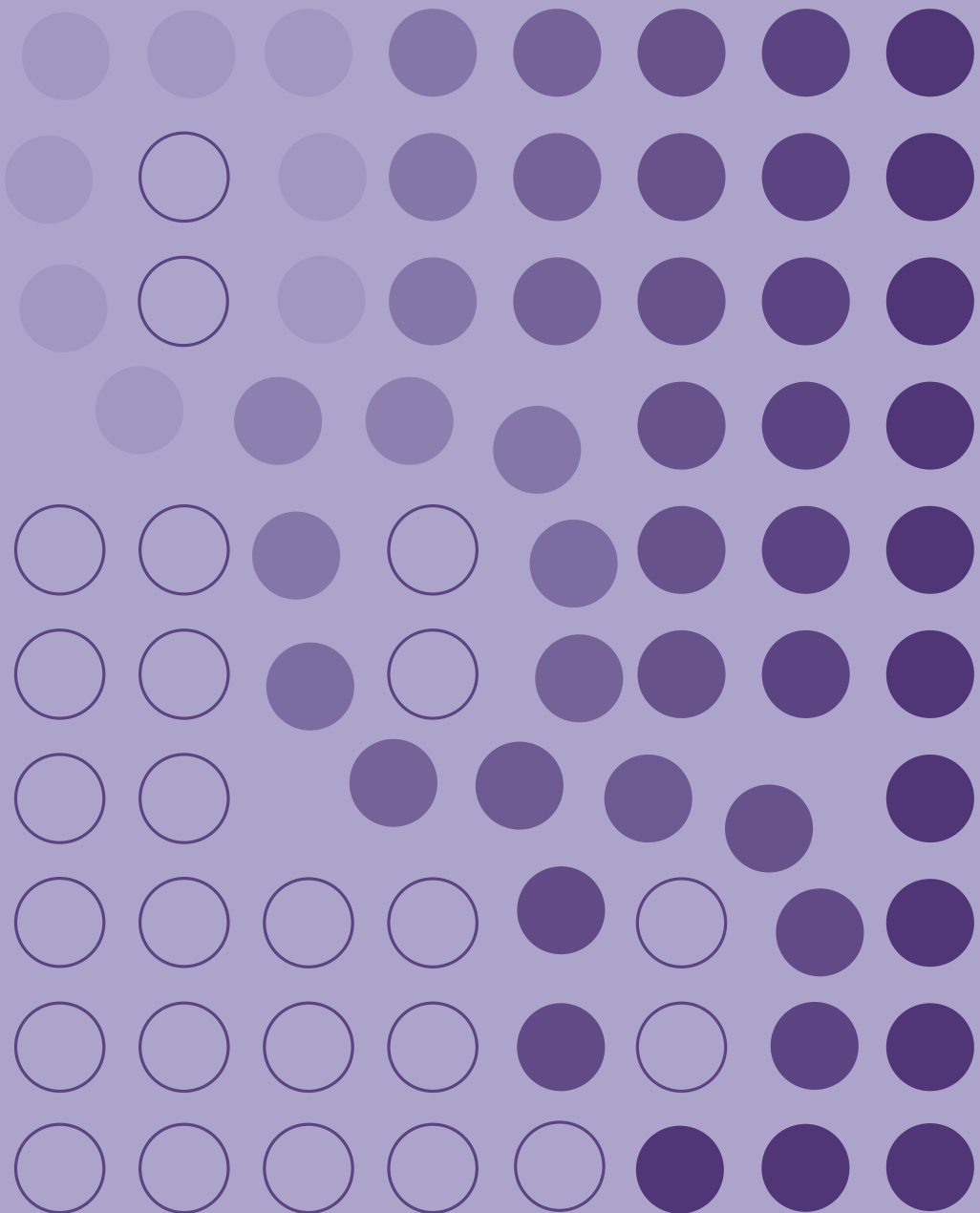
The development of a high-speed, high performance liquid chromatography (HPLC) method for the determination of concentrations of rabeprazole and its metabolite rabeprazole thio-ether in the serum of Caucasian individuals is addressed in **chapter 7**. This fast technique was used because of the unstable 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 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%). 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 optimalization 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 uurs 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 farmacogenetica[1] 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 allel. 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 fentoypes.*

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/*17* of **2/*17* 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 Kaukasische 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 Kaukasische 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 maagzuur-gerelateerde 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 (Kaukasische) populatie
- onderzoek naar de invloed van CYP2C19-varianten op de farmacokinetiek en farmacodynamiek van PPIs bij Kaukasische 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* allel 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*-allel laat zien dat dit allel 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 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 liep 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 extensive metabolizers, zeven heterozygote extensive 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 rabeprazolgroep. 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 gebruikt 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 stabiliteit van de monsters onderzocht. De intra- en inter-dagvariatie, opbrengst en stabiliteit 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 gescoord 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 uitkomstmaten 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 PPIs in aflopende volgorde als volgt is: rabeprazol 20 mg > lansoprazol 30 mg > omeprazol 20 mg > pantoprazol 40 mg > esomeprazol 40 mg. Voor de lagere doseringen geldt dat omeprazol 10 mg meer beinvloed 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 maagzuurgerelateerde aandoeningen.

Dankwoord

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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De verpleegkundigen Bert, Monique, Anne, Rinia, Alieke, Pauline, Petra, Winny, Amarenza, Kitty, unithoofd Merel en iedereen van het secretariaat. Ik denk dat ik zo'n 2000 keer op en neer gelopen ben van de ziekenhuisapotheek naar de poli MDL. Gelukkig stond er altijd koffie voor me klaar. Dank voor jullie hulp en de leuke gesprekken tijdens de bloedafnames. En het MDL lab van het ErasmusMC: Jan Francke en collega's. Ademtesten konden altijd tussendoor en sneller dan verwacht kreeg ik de uitslagen door. Dank voor de prettige samenwerking. Willem-Jan Hofsté: ruim honderd venflons zijn door jou vakkundig geprikt. Het was iedere keer weer prettig als ik probleemloos bloed af kon nemen.

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Den Haag, juli 2010

Nicole

List of publications related to this thesis

- Hunfeld NG, Geus WP, Kuipers EJ. Systematic review: Rebound acid hypersecretion after therapy with proton pump inhibitors. Aliment Pharmacol Ther. 2007;25:39-46.

- Hunfeld NG, Mathot RA, Touw DJ, van Schaik RH, Mulder PG, Franck PF, Kuipers EJ, Geus WP. Effect of *CYP2C19*2* and **17* mutations on pharmacodynamics and kinetics of proton pump inhibitors in Caucasians. Br J Clin Pharmacol. 2008;65:752-60.

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- Hunfeld NG, Touw DJ, Mathot RA, Mulder PG, van Schaik RH, Kuipers EJ, Kooiman JC, Geus WP. A comparison of the acid-inhibitory effects of esomeprazole and pantoprazole in relation to pharmacokinetics and CYP2C19 polymorphism. Aliment Pharmacol Ther. 2010;31:150-9.

- Hunfeld NG, Touw DJ, Kuipers EJ, van Schaik RH. Genetic polymorphisms of CYP2C19 in the Dutch population. Submitted.

- Hunfeld NG, de Goede AL, Grandia L, Kuipers EJ, Touw DT. Systematic review: the influence of CYP2C19 polymorphism on the acid-inhibitory effects of proton pump inhibitors. Submitted.

- Hunfeld NG, Touw DJ, Mathot RA, Mulder PG, van Schaik RH, Kuipers EJ, Kooiman JC, Geus WP. A comparison of the acid-inhibitory effects of esomeprazole and rabeprazole in relation to pharmacokinetics and CYP2C19 polymorphism. In progress.

Over de auteur

Nicole G.M. Hunfeld werd geboren op 28 januari 1978 te Waalwijk. Zij is samen met Maayke, Stephanie en Pieter opgegroeid in Kaatsheuvel. In 1996 behaalde zij het gymnasium diploma aan het Dr. Mollercollege te Waalwijk, waarna zij aanving met de studie Farmacie aan de Universiteit Utrecht. Tijdens de studie Farmacie voerde zij een onderzoeksproject uit naar de effectiviteit van zenuwblokkade door injectie van microsferen gevuld met tetrodotoxine en bupivacaine in ratten. Dit vond plaats bij de onderzoeksgroep van Prof.dr. B. Langer aan het Massachusetts Institute of Technology, in samenwerking met Harvard Medical School te Boston.
Daarnaast verrichtte zij een epidemiologisch onderzoek naar het optreden van hoest als bijwerking van ACE-remmers en AT-2-antagonisten in de openbare apotheek, begeleid door Drs. M.Th.P.J. Voesten en Prof.dr. B. Leufkens. In februari 2003 volbracht zij het apothekersexamen en in dezelfde maand is zij begonnen als projectapotheker bij de Apotheek Haagse Ziekenhuizen te Den Haag, in het klinisch farmaceutisch en toxicologisch laboratorium. In juni 2003 is zij vanuit de Apotheek Haagse Ziekenhuizen begonnen met wetenschappelijk onderzoek naar de genetica, kinetiek en dynamiek van proton pomp remmers, hetgeen geresulteerd heeft in dit proefschrift.
Dit onderzoekstraject vond plaats op de afdeling Maag-, Darm- en Leverziekten van het HagaZiekenhuis en het Erasmus Medisch Centrum onder supervisie van Prof.dr. E.J. Kuipers, Dr. W.P. Geus en Dr. D.J. Touw. In oktober 2007 is zij gestart met de opleiding tot ziekenhuisapotheker in het Haga Ziekenhuis en bij de Apotheek Haagse Ziekenhuizen te Den Haag (opleider Drs. B.H. Graatsma, Apotheek Haagse Ziekenhuizen). Per 1 januari 2011 zal zij werkzaam zijn als ziekenhuisapotheker in het Erasmus Medisch Centrum te Rotterdam.

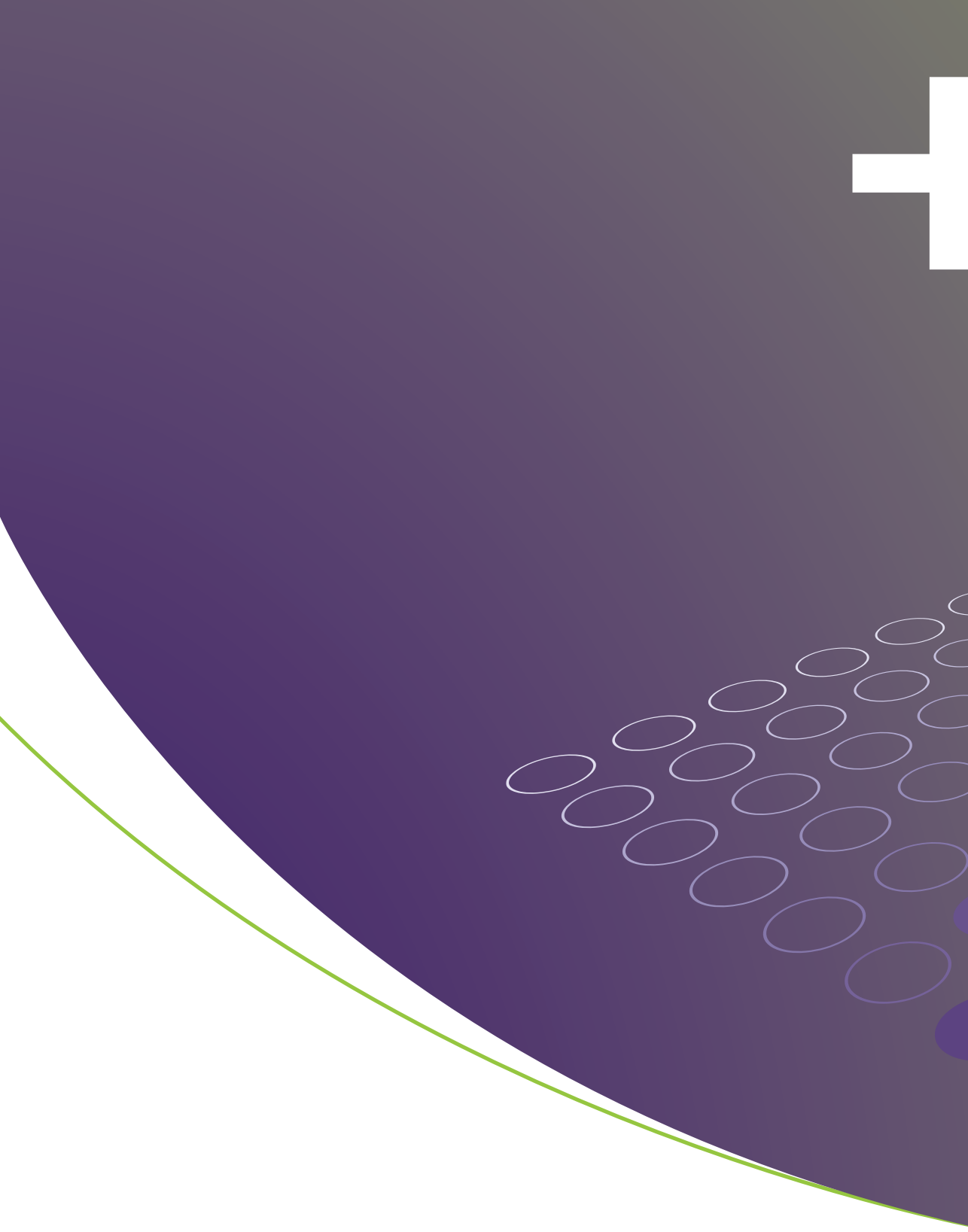
HagaZiekenhuis van Den Haag

Het HagaZiekenhuis van Den Haag heeft vier locaties: Leyweg, Sportlaan, Juliana Kinderziekenhuis en buitenpolikliniek Wateringse Veld. Met ruim 700 bedden, 3.500 medewerkers en 210 medisch specialisten is het HagaZiekenhuis het grootste opleidingsziekenhuis in de Haagse regio.

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
De HagaAcademie, het opleidingsinstituut van het HagaZiekenhuis, biedt alle faciliteiten die nodig zijn voor opleiding, onderwijs en onderzoek en heeft de erkenning voor 23 medisch specialistische opleidingen, waarvan veertien poortspecialisten. Jaarlijks komen ruim 600 arts-assistenten en co-assistenten om een deel van hun opleiding in het HagaZiekenhuis te volgen. De HagaAcademie faciliteert ook in opleidingen van paramedici, (gespecialiseerd) verpleegkundigen en andere opleidingen.

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



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