Pharmacodynamic Modelling of Irreversible and Reversible Gastric Proton Pump Inhibitors

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Abstract

Acid related diseases like GERD, duodenal-and gastric ulcers and H. Pylori-positive peptic ulcer disease are primarily managed by reducing gastric acidity. Irreversible proton pump inhibitors (PPIs) inhibit gastric acid secretion effectively throughout the day by irreversibly inhibiting the gastric proton pump, H+, K+-ATPase, in the parietal cells. Reversible gastric proton pump inhibitors are under development, but have not yet reached clinical use.

The pharmacokinetic/pharmacodynamic (PK/PD) relationships of these compounds are nonlinear, with a delay in the effect-time profile compared to the plasma concentration-time course. PK/PD-modelling was used to characterize and quantify the pharmacological effect with regard to onset, intensity and duration of effect. Models based on functional data, that discriminate between drug-and system-specific parameters, were developed.

In general, the plasma concentration-time course for each individual was approximated by linear interpolation between time-points and served as input into the pharmacodynamic models. A turnover model of irreversible inhibition of gastric acid secretion by omeprazole in the dog described the data well. The model was challenged and found to be robust under different experimental conditions. This model could predict the effect following different exposure of omeprazole and following different histamine provocation. Different fitting approaches (naïve pooling, standard two-stage and nonlinear mixed effects modelling) were compared and resulted in similar parameter estimates. For the reversible inhibitors, a kinetic binding model was finally selected. With a binding model the delay in the effect-time profile is explained by prolonged binding to the enzyme.

Use of these results in drug development can be helpful with regard to selection of drugs for further development and to predict the first clinical dose.

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


IV. Äbelö, A., Holstein, B., Andersson, M., Karlsson, M O. Application of a combined effect-compartment and binding model for gastric acid inhibition of AR-HO47108, a reversible gastric acid proton pump inhibitor, and its active metabolite AR-HO47116, in the dog. (manuscript)

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Abbreviations

- **AOP**: Acid output
- **AS**: Acid secretion
- **ATP**: Adenosine triphosphate
- **Baseline**: Histamine-induced baseline
- **C\text{c}**: Concentration in the biophase
- **C\text{p}**: Plasma concentration
- **CL**: Clearance
- **CL_{M}**: Clearance of metabolite
- **Const**: Constant relating fraction activated enzyme to the observed effect
- **E_{A}**: Fraction activated enzyme
- **E_{I}**: Fraction inactivated enzyme by drug/metabolite
- **EC_{50}/IC_{50}**: Concentration at 50 % of maximal effect/inhibition
- **E_{\text{max}}/I_{\text{max}}**: Maximum drug-induced effect/inhibition
- **F**: Bioavailability
- **GERD**: Gastroesophageal reflux disease
- **H^{+}, K^{+}\text{-ATPase}**: Hydrogen, potassium adenosine triphosphatase
- **k**: First order plasma concentration elimination rate constant
- **k_{1}**: First order rate constant for the transfer of enzyme from the pool to the response compartment
- **k_{1,\text{his}}**: Second order rate constant for the transfer of enzyme from the pool to the response compartment/per histamine/concentration unit
- **k_{2}**: First order rate constant for the transfer of enzyme from active to resting state
- **k_{a}**: Absorption rate constant
- **K_{D}**: Equilibration dissociation constant of the enzyme-drug complex
- **k_{e0}**: First order elimination rate constant out of the effect compartment
- **k_{\text{turn}}**: Turnover rate
- **k_{\text{off}}**: Dissociation rate constant of the enzyme-drug complex
- **k_{\text{ome}}**: Second order rate constant for the
irreversible inhibition of enzyme by omeprazole

\( k_{in} \)  
Association rate constant of the enzyme-drug complex

\( k_{out} \)  
Fractional turnover rate

PK  
Pharmacokinetics

PK/PD  
Pharmacokinetics/pharmacodynamics

PPI  
Irreversible proton pump inhibitor

SEM  
Standard error of the mean

\( t_{1/2} \)  
Plasma concentration half-life

\( t_{lag} \)  
Lagtime

\( V \)  
Volume of distribution

\( V_M \)  
Volume of distribution of metabolite
1. Introduction

By measuring the pharmacological effect in conjunction with the plasma concentration simultaneous pharmacokinetic and pharmacodynamic modelling (PK/PD-modelling) can be carried out (Figure 1).

Figure 1. Schematic illustration of the dose-concentration-effect relationship of drugs. Adapted from Meibohm and Derendorf, 1997.1

PK/PD-modelling involves finding mathematical models that describe the dose-concentration-effect-time relationships of drugs. Such models allow estimates of potency ($EC_{50}/IC_{50}$) and intrinsic activity ($E_{max}/I_{max}$) to be derived on the basis of concentration (exposure) instead of dose.2,3 In addition; the change in the effect with time has to be taken into account, if equilibrium has not been established between concentration and effect. The model should be challenged with respect to its performance following different doses and different rates and routes of drug administration.

The general objective of this thesis is to characterize the relationships between the plasma concentration of gastric proton pump inhibitors and gastric acid inhibition using PK/PD-modelling.
1.1. Mechanism and regulation of gastric acid secretion

Acid secretion from the gastric mucosa is a property of the parietal cell, and depends on the activity of the gastric H⁺, K⁺-ATPase (Figure 2).⁴

![Figure 2. Schematic presentation of a secreting parietal cell. The parietal cells are located deep in the gastric mucosa. In the resting parietal cell the H⁺, K⁺-ATPase is inactive and present largely in tubular vesicles in the cytoplasm of the cell. Stimulation of the parietal cell occurs via the acetylcholine (M₃), gastrin (CCKₐ) and/or histamine (H₂) receptors on the basolateral membrane and results, via second messengers, in a transfer of inactive H⁺, K⁺-ATPase to the apical membrane of the cell. H⁺, K⁺-ATPase transports protons from the cytoplasm into the secretory canaliculi in exchange for potassium. Chloride ions enters the secretory canaliculi from the cytoplasm by a passive transport mechanism resulting in the overall secretion of HCl.⁵ PPIs and reversible proton pump inhibitors are weak bases and concentrate easily in the acidic space of the parietal cell. Within the cell, the PPI is transformed to a cyclic sulfenamide, the active principle, and binds irreversibly to H⁺, K⁺-ATPase.⁶

The parietal cells are located in the oxyntic glands in the fundus and corpus of the stomach. Stimulation of the parietal cell occurs via the acetylcholine, gastrin, and/or histamine receptors on the basolateral membrane of the cell. The stimulation involves relocalisation of the H⁺, K⁺-ATPase from tubular membranes in the cytoplasm to the apical, or canalicular, membrane, where it becomes associated with a K⁺ and Cl⁻ conductance, and hence activated.⁷,⁸ When localised in the tubule membrane, separate from the canaliculus, the enzyme is inactive due to inadequate K⁺ access to the luminal face of the
pump. The enzyme at the apical membrane transports one proton out of the cell in exchange for one potassium at the expense of one molecule of ATP. To be able to generate the energy required for the ion transport the parietal cells are rich in mitochondria. Stimulation of the parietal cell also causes increase in the movement of Cl\textsuperscript{−} across the membrane into the canalicular lumen, where it mixes with H\textsuperscript{+} and forms HCl. Upon stimulation large quantities of nearly isotonic (160mM) HCl fluid can be produced.

Basal secretion in man is highly variable and shows a circadian rhythm characterized by a high rate in the evening and a low rate in the morning. There is a great species variation in basal acid secretion. In dogs it is 1 % of the maximal capacity, whereas in rats and man it is 30 % and 10 %, respectively.

The acidity is regulated through different mechanisms that can be divided into four phases, the cephalic phase, the gastric phase, intestinal phase and the interdigestive or basal state. In the basal state acid is secreted in the absence of an external stimulus. The cephalic phase is initiated by the thought, sight, smell and taste of food. The vagal nerve mediates the cephalic stimulus to the stomach and gastrin is released in this phase. The gastric phase is initiated by stomach distension and the presence of certain chemicals in the gastric lumen. Food is the most important stimulus of gastrin release, which is the major mediator of the gastric phase. The intestinal phase is initiated by the presence of certain chemicals (amino acids) in the intestine. Gastrin does not seem to be important in this phase of acid secretion. The presence of acid in the duodenum stimulates the release of hormones e.g. secretin, somatostatin, which inhibits acid secretion by reducing gastrin release. The major known mechanism for stimulation of acid secretion is through stimulation of the enterochromaffin-like cells (ECL-cells), by gastrin and acetylcholine, to release histamine. The major of the peripheral mechanisms for regulation of acid secretion is the plasma gastrin level. Gastrin secretion from the G-cell of the gastric antrum starts to decrease when intragastric pH falls below 3.5. The plasma gastrin level is elevated by food in the antrum and an intragastric pH above 3 in the presence of food can lead to hypergastrinemia.

1.2. Gastric acid related disorders and pharmacological control of acid secretion

The major roles of gastric acid is to activate the proteolytic pepsin enzymes, which are required for digestion of food and to kill bacteria within the stomach. In addition to acid producing parietal cells, the oxyntic glands contain cells that produce mucus, which protects the cell surface from the
acidic contents of the lumen. The gastro duodenal mucosa may be injured if there is an imbalance between aggressive factors (acid and pepsin) and defense factors (mucosal resistance). Ulcers in the stomach and in the lower part of esophagus and in the duodenum may then occur. Although acid is essential for ulcer formation, many patients with ulcers have normal or even subnormal rates of acid secretion. However, reducing the level of acid secretion promotes healing and tends to prevent the recurrence of ulcers.

Genetic susceptibility, drugs, alcohol, bile salts, and excessive secretion of acid and pepsin may contribute to ulcer formation. The major factor, however, may be the presence of Helicobacter pylori, which is present in the stomach of the majority of the patients with ulcers or gastritis. Suppression of this bacterium with antibiotics often leads to healing of the damaged mucosa. Gastro-esophageal reflux disease (GERD) is a condition that includes both symptoms and esophageal damage occurring as a consequence of reflux of gastric contents into the esophagus. The severity of the disease correlates with the degree and duration of esophageal acid exposure. Heartburn is the most common symptom in GERD patients.

Acid related diseases like GERD, duodenal-and gastric ulcers and Helicobacter pylori-positive peptic ulcer disease are primarily managed by reducing gastric acidity. There are several ways of interfering with acid secretion, antacids, receptor antagonists and proton pump inhibitors. Antacids are compounds that can buffer and neutralize the gastric content. Another approach to control acid secretion is through interference with receptors on the acid secreting parietal cell (Figure 2). The discovery and development of H2-antagonists in the early 1970s revolutionized the treatment of acid related diseases. These compounds can effectively reduce acid secretion through a competitive interaction with the H2-receptor on the parietal cell. Studies in man have shown that histamine-H2-antagonists can inhibit acid secretion stimulated by histamine, gastrin and vagal activation and ingestion of food. However, as the parietal cell also has receptors for acetylcholine and gastrin, the inhibition of acid secretion via the H2-receptor can also be overcome by stimulation via these receptors. In addition, some degree of tolerance develops. Irreversible proton pump inhibitors (PPIs) do inhibit acid secretion throughout the day, and can overcome meal stimulated acid secretion. They are used in combination with antibiotics in the treatment of Helicobacter pylori-positive ulcers. In patients with GERD both healing and control of symptoms have been shown to be directly correlated to inhibition of intragastric pH of the 24 h period. Therefore the PPIs are superior to the other drugs in the treatment of GERD. A drawback with the PPIs is that the full effect of these drugs is not reached until 3 to 5 days after starting the treatment. One of the aims with the development of the
reversible proton pump inhibitors is to have a faster onset of action. However, none of these have yet reached clinical use.

1.3. Studied drugs

Omeprazole (5-methoxy-2[[4-methoxy-3,5-dimethyl-2-pyridinyl]-methyl][sulfanyl]-1H-benzimidazole) is a substituted benzimidazole with a pKa of 4.0 and a molecular weight of 345 (Figure 3).

\[
\text{Omeprazole} \quad \text{H 335/25}
\]

\[
\text{AR-H047108} \quad \text{AR-H047116}
\]

\[
\text{H 335/25 (3-buturyl-4(2-methylphenylamino)-8-(2-methylsulfinylethoxy)-quinoline is a reversible gastric acid proton pump inhibitor. It is a weak base, pKa=6.5, with a molecular weight of 410 (Figure 3).}
\]

\[
\text{AR-H047108, an imidzopyridine (2,3-dimethyl-8-(2-ethyl-6-methyl-benzyl-amino)-imidazo[1,2-a]pyridine-6-carboxamide), with a molecular}
\]

\[
\text{Figure 3. Chemical structures of omeprazole, H 335/25, AR-H047108 and AR-H047116.}
\]
weight of 336 (Figure 3), and its active metabolite AR-H047116 (8-(2-ethyl-6-methylbenzylamino)-3-hydroxymethyl-2-methylimidazo[1,2-a]pyridine-6-carboxamide), with a molecular weight of 352 (Figure 3), are reversible gastric acid pump inhibitors. The compounds are weak bases with pKa=5.9 and pKa=5.4, respectively.

1.4. Mechanism of action of proton pump inhibitors

The irreversible proton pump inhibitors (PPIs) block the final common pathway leading to acid secretion (Figure 2). Due to the weak base properties of PPIs (pKa~4), the protonated form concentrates in the acidic space of the canaliculus (pH=1). In the acidic environment, the protonated form is transformed into a cationic sulfenamide, which covalently binds to accessible cysteines on the H⁺, K⁺-ATPase and thereby inhibits its activity. The rate of the acid catalyzed transformation to the active form is probably slower in the tubulovesicles because of its dependence of acidity, which may explain why only the enzymes present in the secretory canaliculus are inhibited. In addition, only the pumps in the canalicular membrane form HCl. The membranes of the tubulovesicles lack the essential potassium conductance and, therefore, there will be no proton exchange.

The PPIs have a long duration of action, because recovery of acid secretion following covalent inhibition and repeated once-a-day dosing primarily depends on de novo synthesized enzymes. Reduction of the disulfide between pump enzyme and inhibitor may, to some extent, contribute to the recovery of acid secretion. The plasma half-life of the PPIs is only about 1-2 hours, whereas the half-life of H⁺, K⁺-ATPase in rat and of acid secretion inhibition in man is 50 hours.

A number of heterocyclic compounds have been reported to belong to a new class of proton pump inhibitors. Although structurally different, they act by a common mechanism of action. They all contain protonable nitrogens and are weak bases and concentrate therefore in the acidic compartment of the parietal cell. They inhibit acid secretion by binding to H⁺, K⁺-ATPase from the lumenal side in a K⁺-competitive and reversible manner. In contrast to the PPIs, the reversible inhibitors are active in the absence of stimulated acid secretion and should therefore produce a less variable onset of the effect, with steady state inhibition achieved with the first dose. Furthermore, as the drugs bind reversibly, the inhibitory action will follow the plasma concentration closely. They should therefore produce a more reproducible and short-lived effect. However, a delay in the effect-time profile compared to the plasma concentration-time profile was observed for the compounds studied in this thesis.
2. Aims of the thesis

The general objective of this thesis was to characterize the relationships between the plasma concentration (exposure) of gastric proton pump inhibitors and gastric acid inhibition using pharmacokinetic-pharmacodynamic modelling. This included:

- Development of a turnover model describing the inhibitory effect of omeprazole on gastric acid secretion that separates drug-specific and system-specific parameters.

- Characterization of the histamine effect on acid secretion in dogs.

- Characterization of the dynamics of H 335/25, AR-H047108 and AR-H047116, three reversible proton pump inhibitors, with respect to onset, intensity and duration of response.

- Determination of the relative potency in vivo of AR-H047108 and its active metabolite, AR-H047116.
3. Methods

3.1. Experimental procedure

The studies in dogs were approved by the Laboratory Animal Ethics Committee of the administrative court of appeal in Gothenburg.

In all dog experiments, gastric acid secretion was stimulated with histamine. Gastric juice was collected during the period of histamine stimulation, generally in 30-min fractions. Depending on purpose and dog model, the test compound was given either at different times before, or during, the period with histamine infusion. The acidity of the gastric juice was determined by titration to pH 7.00 with NaOH, 0.100 mol/l. Acid secretion (expressed as mmol H⁺) was calculated from sample acidity and weight.

The experiments in the dog were conducted in animals provided with a conventional gastric fistula for collection of gastric juice and with a duodenostomy for intraduodenal drug administration (Paper I) or in dogs equipped with a cannulated Heidenhain pouch (HP) for the sampling of gastric juice (Papers II, III and IV). The animals were trained in Pavlov stands.

In the gastric fistula dog the whole stomach is drained. Therefore, test compound cannot be given orally during collection of gastric juice. Instead, the substance has to be given before the gastric cannula is opened, allowing enough time for the compound to be emptied into the intestine. In the Heidenhain pouch dog model, on the other hand, a part of the stomach is tied off and a pouch is formed, which is drained to the outside through a cannula. This allows oral dosing during collection of gastric juice from the pouch and assessment of both onset and dissipation of the effect.

The histamine dose was individually selected to induce stable acid secretion. Based on histamine dose-response studies performed in each dog, a histamine dose was selected, that produced maximal acidity (~150 mmol H⁺/L) but about 70 % of maximal acid output. The stimulation period with histamine is limited to 6.5 hours; this allows assessment of inhibitory effects up to 5 hours after dose (paper I and II). In order to collect PD-data from a later time period after dose, separate experiments were done in each dog, each time with a different interval between dosing and testing, and with a
wash-out period between successive doses of active compound (papers III and IV).

In paper I, the histamine stimulated baseline values were determined by placebo experiments and in order to take placebo effect into consideration, the percentage drug-induced change in acid secretion from the baseline was calculated for each dog at each time point. In paper II and IV the actual acid secretion was modelled instead of the percentage drug-induced change in acid secretion from the baseline. In paper III, the histamine induced baseline response was modelled simultaneously with active treatment. Blood samples for the determination of drug in plasma were collected in all experiments.

3.2. Healthy study subjects and study protocol

The study (paper II) was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty of the University of Gothenburg. Written informed consent was received from all subjects prior to participation.

Eight males with a median age of 25 years and, a median weight of 76 kg were included in the study. They were all Helicobacter pylori-negative. The study was performed as an open study with oral administration of single doses of 40-800 mg of H 335/25.

After an overnight fast a double-lumen nasogastric tube, to which a thin polyethylene catheter was attached, was introduced and its tip placed in the fundus. The stomach was continuously perfused with a solution of phenol red in 0.9 % NaOH. Gastric contents were collected in 15-min samples by aspiration. After 2x15-min collections of basal secretion, pentagastrin was administered intravenously and stimulated secreted acid was collected for another four 15-min periods. Thereafter, the perfusion and aspiration was stopped and the appropriate oral dose was administered. Forty minutes after intake of the oral dose, aspiration was resumed and the stomach emptied during 5 min. Forty-five minutes after dose intake, perfusion restarted and aspiration of gastric juice continued with 15-min intervals for 3.25 h. Volume and pH were recorded in each sample and the acid concentration determined by titration to pH 7.00 with 0.1 M NaOH. Blood samples were taken at selected intervals over 24 h subsequent to drug administration in all experiments.
3.3. Bioanalytical assays

The analytical procedure in papers I and III for the determination of unchanged omeprazole in plasma was based on extraction of the plasma samples at pH 7.0 with methylene chloride, followed by liquid-solid chromatography. The compound was eluted with a mixture of methanol and NH₄OH and methylene chloride. The elution profile was monitored by ultraviolet detection and compared with standard concentrations of omeprazole. The fraction of the eluate corresponding to [¹⁴C]omeprazole was counted by liquid scintillation.²⁷,²⁸

In paper II, H 335/25 and the internal standard were extracted at pH 8-9 from plasma into isohexane containing 40 % dichloromethane. The organic phase was evaporated and the extract was redissolved in acid and washed with hexane. An aliquot was injected onto an LC-column (Kromasil 100-5C8, 5 µM, 150x4.6 mm, obtained from Hichrom Ltd., England). The mobile phase contained 45 % acetonitrile and 10 % aqueous acetate buffer 0.5 mol/l, pH 4.2 (flow-rate 1.0 ml/min). Retention times were 5 and 8 min for H 335/25 and the internal standard, respectively. The compounds were detected in the eluate using UV at 252 nm. The limit of quantification was 15 nmol/l (CV<20 %) and accuracy within 85-115 %. The daily calibration of the analytical system was done with single concentration plasma standards. The intra-day CVs of single concentration plasma standard sets (n=9-12) for H 335/25 in the dog study was in the range 1.7-2.8 %. The reproducibility of the method, estimated by daily quality control samples (n=2) gave a mean level of 103.2 % (range 102.0-104.5 %) of nominal value. The intra-day CVs of single concentration plasma standard sets (n=10) for H 335/25 in the human study was in the range 1.4-2.8 %. The reproducibility of the method was controlled by daily quality control samples (n=2), which gave a mean level of 97.2 % (range 96.1-98.4 %) of nominal value.

In paper IV, the concentration of AR-H047108 and AR-H047116 in blood plasma was determined by normal-phase liquid chromatography and fluoroscence detection. Analytes were separated on a 250 x 4.0 mm Superspher SI 60, 4 µm particle size column (E. Meck, Germany), maintained at 27 °C. The mobile phase consisted of 0.7 % 1 M perchloric acid, 2.3 % methanol and 9.4 % 2-propanol in dichloromethane and was pumped isocratically at a flow-rate of 1.0 ml/min. As internal standard (IS) a structural analogue, AR-H044277, to the parent drug was used. The retention times for AR-H047108, IS and AR-H047116 were about 7.0, 10.5 and 12.5 min, respectively.

Frozen plasma samples were thawed at room temperature, mixed and centrifuged 5 min at 3000 rpm. Mixture of 500 µl plasma, 50 µl internal standard solution and 50 µl carbonate buffer pH=9.7 (I=1.25) giving a final
pH of 9-10, was extracted with 1.0 ml 2 % 1-butanol in dichloromethane. After centrifugation the aqueous phase was aspirated and 150 μl of the organic phase was injected onto the chromatographic column.

Daily calibration of the analytical system was done with single concentration plasma standards. The linearity of the method was estimated by running a full standard curve, comprising 8 concentrations and at least triplicate at each concentration, once a month. The found concentrations were calculated relative to plasma standards with concentrations similar to the ones used in the daily calibration. The limit of quantification (LOQ) was set at 2.0 nmol/L (CV<20%) for AR-H047108 and 5.0 nmol/L (CV<20%) for AR-H047116. Extraction recovery for the two analytes and IS from dog plasma was about 95 %. The repeatability of the method, the intra-day coefficient of variation (CV), was 1.7 % for AR-H047108 and 1.4 % for AR-H047116 at 280 and 640 nmol/L of dog plasma, respectively. The reproducibility or between-day variability of the method, estimated from daily quality control samples (n=18) gave a mean level of found concentrations of 101.9 % (CV 3.2 %) for AR-H047108 and 99.6 % (CV 1.1 %) for AR-H047116 at 310 and 270 nmol/L of plasma, respectively.

3.4. Data analysis

The plasma concentrations of omeprazole and the acid secretion were modelled sequentially in paper I. Compartmental models were applied to describe the plasma kinetics of omeprazole. The final parameter estimates of the kinetic model were then fixed and served as input to the pharmacodynamic models. Ordinary least-square regression (WinNonlin Professional, Pharsight Corporation, Mountain View, CA) was applied for all models. The goodness of fit was assessed by residual analysis. Initial parameter estimates were derived graphically. For a basic turnover model, with for example drug action on the production of response, an initial estimate of \( k_{out} \) is obtained from the slope of the onset of the response-time curve. From the intercept of the effect axis, the baseline value can be obtained. \( EC_{50} / IC_{50} \) and \( E_{max} / I_{max} \) can be obtained graphically from inspection of an effect versus log concentration plot.

In papers II, III and IV, the plasma concentration-time profile for each individual was approximated by linear interpolation between time points up to the last time point and served as input in the pharmacodynamic models. The individual plasma concentration half-life was estimated by log-linear regression analysis and used in the extrapolation of the plasma concentration beyond the last plasma sample. All modelling was done in NONMEM, version V, level 1.1. Individual parameter values were obtained by using
Bayesian estimation. Model adequacy was evaluated by graphical means\textsuperscript{31} as well as by the objective function value (OFV), which is proportional to minus two times the LogLikelihood. For hierarchical models, the difference in objective function values is approximately Chi-square distributed and it can therefore be used for comparison between models. For a one-parameter difference between models, 3.84 correspond to a p-value of 0.05. For non-hierarchical models with the same number of parameters, the model providing the lower objective function value is favored, but no p-value can be obtained.
4. Modelling of the relationship between plasma concentration and effect

The purpose of modelling preclinical in vivo data is to quantify and to gain a better understanding of the underlying mechanism of the drug effect. This is important in order to make useful predictions of the effect after different rates and routes of drug administration, and from animal to man. The models used are mathematical models. The most common model is the $E_{\text{max}}$-model (equation 1), which is derived based on receptor occupancy theory, but often used empirically to describe the effect of drugs for the whole range of concentrations.

$$E = E_0 + \frac{E_{\text{max}} \cdot C}{EC_{50} + C}$$  
(Eq. 1)

$E$ is the observed effect at concentration $C$; $E_0$ is the baseline effect when no drug is present. $E_{\text{max}}$ is the maximal effect and $EC_{50}$ is the concentration producing 50% of maximal drug induced effect. It should be realized, that $E_{\text{max}}$ and $EC_{50}$ are ‘mixed’ parameters that depend on both drug specific (affinity and intrinsic activity) and system specific (receptor density and efficiency of receptor-effector coupling) properties. The system specific properties can change with, for example, disease and age and this should be taken into account in order to make useful predictions of the in vivo situation from in vitro data.

This type of model is valid for the steady state situation or in the case of instantaneous concentration-response equilibration.

The inhibitory effect of cimetidine, a H$_2$-receptor antagonist, on acid secretion has been described by the sigmoidal $E_{\text{max}}$-model. However, for reversible proton pump inhibitors (paper II and IV) counter clockwise hysteresis is observed i.e. there is a delay in the effect-time profile compared to the plasma concentration-time profile. Different standard delay models (link, receptor binding and turnover models), for non-steady state conditions were applied to characterize the relationship between plasma concentration and observed effect for these compounds. All models, applied to the reversible inhibitors, assume that acid secretion is proportional to the
fraction of activated enzymes, $E_A$, and that the total number of enzyme is constant. This should be a reasonable assumption, as the reversible inhibitors does not require acid-catalyzed transformation to be activated.$^{21,24}$ When the enzyme is inactivated reversibly, the acid secretion, $AS$, will decrease:

$$AS = \text{Baseline} \cdot [E_A] \quad \text{(Eq. 2)}$$

Three different models were studied; an effect compartment model$^{39,40}$ (Figure 4), a binding model$^{41,42}$ (Figure 5A) and a turnover model$^{43}$ (Figure 5B). In paper IV, a combined effect compartment and binding model was also applied.

The effect compartment approach assumes that the time-delay in response is governed by the equilibration between plasma and a hypothetical effect compartment \textit{i.e.} the canaliculus of the parietal cell in this case. The effect compartment is linked to the plasma compartment by a first order rate constant, $k_{1e}$, and drug is eliminated from the effect compartment by a first order rate constant, $k_{e0}$. The rate of change of drug and metabolite concentration in the effect compartment can be described by Eq. (3) and Eq. (4):

$$\frac{dC_{e,\text{drug}}}{dt} = k_{1e,\text{drug}} \cdot C_{p,\text{drug}} - k_{e0,\text{drug}} \cdot C_{e,\text{drug}} \quad \text{(Eq. 3)}$$

$$\frac{dC_{e,\text{met}}}{dt} = k_{1e,\text{met}} \cdot C_{p,\text{met}} - k_{e0,\text{met}} \cdot C_{e,\text{met}} \quad \text{(Eq. 4)}$$

Under the assumption that the biophase is sufficiently small not to influence the plasma concentration time-profile, these equations can be reduced to Eqs. (5) and (6):

$$\frac{dC_{e,\text{drug}}}{dt} = k_{e0,\text{drug}} \cdot (C_{p,\text{drug}} - C_{e,\text{drug}}) \quad \text{(Eq. 5)}$$

$$\frac{dC_{e,\text{met}}}{dt} = k_{e0,\text{met}} \cdot (C_{p,\text{met}} - C_{e,\text{met}}) \quad \text{(Eq. 6)}$$

The observed effect was assumed to be related to the biophase concentration by an inhibitory $E_{\text{max}}$ model where the maximal effect is complete inhibition.
of gastric secretion. The estimated structural model parameters were 
*histamine-stimulated baseline*, \( k_{eo} \), and \( EC_{50} \).

![Figure 4. The plasma concentration (\( C_p \)) is “linked” to an effect compartment. The rate constant out of the effect compartment, \( k_{e0} \), determines the rate of equilibration between plasma-biophase. \( k_{le} \) represents the first-order transfer of negligible amount of mass from the plasma compartment to the effect compartment. \( C_e \) represents the concentration in the biophase.](image)

Delay may be explained by factors other than distribution between plasma and the effect site. There may be a delay in the interaction between the drug and its receptor, or in the relationship between receptor occupation and effect. A binding model can be applied when the receptor interaction is prolonged. In paper II and IV it is assumed that a slow dissociation of the drug from the drug-enzyme complex is the rate-limiting process for the time-course of the effect. Furthermore, in paper IV, because the parent compound and metabolite bind to the same enzyme, the effect is determined by competition between the two for the same enzyme (Figure 5A). The rate of change of activated enzyme, \( E_A \), as a function of plasma concentration is described by Eq. (7):

\[
\frac{dE_A}{dt} = -k_{on,drug} \cdot C_p \cdot drug \cdot E_A + k_{off,drug} \cdot E_{t,drug}
\]

\[
- k_{on,met} \cdot C_p \cdot met \cdot E_A + k_{off,met} \cdot E_{t,met}
\]

\( k_{on} \) and \( k_{off} \) are the association and dissociation rate constants of the drug-enzyme complex, respectively. \( E_I \) is the fraction of enzyme reversibly inactivated by the parent compound (\( drug \)) or metabolite (\( met \)).

The rate of change of \( E_I \) can be described by Eq. (8) and (9) for parent compound and metabolite, respectively.
The estimated structural parameters are baseline, \( k_{on} \), and \( k_{off} \). \( KD \) is estimated as \( k_{off}/k_{on} \), and is equal to \( EC_{50} \) at steady-state.

In paper IV, a combination of the above models for effect delay was applied and obtained by replacing \( C_{p,drug} \) and \( C_{p,met} \) in equations 7, 8 and 9 with \( C_{e,drug} \) and \( C_{e,met} \), given in equations 5 and 6, respectively.

A turnover model can be used when the measured response to a drug is produced by indirect mechanisms. The drug is assumed to inhibit or stimulate the production, turnover rate \( k_{in} \), or elimination, fractional turnover rate \( k_{out} \), of the response. The rate of change of the response with no drug present is described in Eq. 10.

\[
\frac{dR}{dt} = k_{in} - k_{out} \cdot R 
\]

(Eq. 10)

Eq. 11 gives the basal response at steady state

\[
R_0 = \frac{k_{in}}{k_{out}} 
\]

(Eq. 11)

In paper II, a turnover model with drug acting as an inhibitor of production of response\(^{43}\), is applied (Figure 5B). It is assumed that the relationship between enzyme and ventricular amount of acid is proportional but not direct. The drug is assumed to inhibit \( k_{in} \), which leads to a decrease of acid secretion.

At equilibrium the three models predict the same relationship between effect and concentration:

\[
AS_{ss} = \frac{Baseline \cdot X}{X + C_{ss}} 
\]

(Eq. 12)
where $X$ is $EC_{50}$ for the effect compartment and indirect response models and $K_d$, which can be calculated as the ratio of $k_{off}$ to $k_{on}$, for the binding model. Also at high values of $k_{on}, k_{out}$ and $k_{off}$, which are for instantaneous processes, the three models would predict the same response and the $EC_{50}$ would equal the equilibration constant $K_d$. For non-instantaneous processes, the three models always provide different effect-time profiles for a given concentration-time profile.

In Paper I two different models for omeprazole action were studied. In both models it was assumed that acid secretion is proportional to the fraction of active enzyme. A turnover model was applied (Figure 5C). In this model, omeprazole acts by irreversibly removing enzyme from the system (i.e. stimulation of $k_{out}$), at a rate proportional to enzyme and omeprazole concentration. Stimulation of $k_{out}$ leads to a decrease in acid secretion.

To be able to describe the biphasic recovery process of omeprazole-induced inhibition, observed in Paper I, the presence of a pool of enzyme in its inactive state was considered. $P$, in Figure 5D represents the contribution of the enzyme pool. This model was used in Papers I and III.

**Figure 5A-D.**

- **A:** A slow dissociation of drug from the drug-enzyme complex (a low $k_{off}$) is assumed to cause the observed effect-delay. $E_A$ represents active enzymes and $E_D$ and $E_M$ enzymes that are inactivated by drug (D) and metabolite (M), respectively, in a reversible manner (Papers II and IV).

- **B:** The turnover model assumes that the acid secretion ($=R$) is not directly, but proportionally, related to the number of active enzymes. The drug is acting by inhibiting, $I_{(drug)}$, the production of response (Paper II).
C: The turnover model assumes that the acid secretion (=R) is not directly, but proportionally, related to the number of active enzymes. The drug is acting by stimulating, S(ome), the loss of response. \( k_{ome} \) is the second order rate constant for the irreversible binding of omeprazole to enzyme (Paper I).

D: In the non-secreting parietal cell, the enzymes are inactive and located in the cytoplasm of the parietal cell, represented by \( P \) in the model. The acid secretion is activated via stimulation of \( k_1 \) by histamine. The stimulation results in a transfer of enzymes from the pool to the apical membrane of the parietal cell, represented by \( R \). The acid secretion is assumed to be proportional to the amount of activated enzyme in \( R \). Omeprazole is acting by stimulating, S(ome), the loss of response. \( k_{ome} \) is the second order rate constant for the irreversible binding of omeprazole to enzyme (Papers I and III).
5. Results

5.1. Pharmacokinetics of all compounds
In paper I, the published data in the dog that were used that referred to response during a relatively short period of 5 h post dose and to response every 24 h for 4 days post dose. In the short-term experiments, omeprazole was administered as intravenous infusions of different durations on six different occasions. In the long-term experiments intraduodenal single doses were administered on four occasions. A one-compartment model was applied to describe the plasma concentrations in the short-term experiments and the mean (n=2) of the individual estimates of the pharmacokinetic parameters are shown in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Infusion order</th>
<th>Infusion time (min)</th>
<th>Rate of infusion (nmol/kg, min)</th>
<th>Total dose (nmol/kg)</th>
<th>V (l)</th>
<th>k (min⁻¹)</th>
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<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>22.5</td>
<td>0.5</td>
<td>81</td>
<td>0.27</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>277.5</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>22.5</td>
<td>2</td>
<td>323</td>
<td>0.25 (8)</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>277.5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>22.5</td>
<td>2</td>
<td>45</td>
<td>0.34</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>277.5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>22.5</td>
<td>8</td>
<td>1290</td>
<td>0.24</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>277.5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>22.5</td>
<td>8</td>
<td>180</td>
<td>0.30 (8)</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>277.5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>22.5</td>
<td>2</td>
<td>180</td>
<td>0.35 (9)</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>134.5</td>
<td>1</td>
<td></td>
<td></td>
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<td>III</td>
<td>143.0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the long-term experiments, pharmacokinetic data were available for one dose (0.25 μmol/kg) and a one-compartment model with first order
absorption was fitted to mean concentrations (n=3) presented in Larsson et al. 1983. The kinetics was assumed to be linear between doses and plasma concentration curves for the four doses used in the PD study were predicted (Figure 6). Simulated plasma concentration-time curves for two of the short-term experiments (experiments 4 and 5 in Table 1) are also shown in the figure as these were used in the simultaneous model fitting of response data.

![Figure 6. Simulated plasma concentration-time curves for the two short-term experiments 5 and 4 (corresponding to total doses of i.v. infusions of 0.18 and 1.29 µmol/kg, respectively) and the four long-term experiments that were used in the model fitting (0.5-5 µmol/kg i.d.).](image)

In papers II and III, the plasma concentration-time curves for each individual was approximated by linear interpolation between time points and served as input to the pharmacodynamic models. H335/25 (paper II) was administered as single oral (0.75, 1.5, 3, and 6 µmol/kg) and intravenous (1 and 2 µmol/kg) doses to dogs (n=4) and blood samples for determination of drug in plasma were collected in all experiments. Peak plasma concentration was reached at 0.6-0.9 h and the half-life was approximately 1.3 h (Figure 7).
In the human dose escalation study, eight healthy subjects received oral single doses of H 335/25 (40, 80, 150, 300, 500 or 800 mg). Each dose was given to two subjects and each subject received one or two doses of study drugs. Peak plasma concentration was reached within 1 h and the half-life was approximately 1.5 h (Figure 8).

In paper III, four different experiments with omeprazole were carried out (n=4). In experiments 1-3, a total dose of 0.81 µmol/kg was infused during 3 h. In the 4th experiment, a dose of 0.18 µmol/kg was infused for 22.5 min. The obtained plasma concentration-time profiles are shown in Figure 9.
Figure 9. Plasma concentrations of omeprazole after intravenous infusion of omeprazole sodium in the Heidenhain pouch dog (Mean±SE, n=4) (paper III).

The mean plasma concentration at the end of the 22.5 min infusion was similar in Exps 1-4, with mean values of 554-563 nmol/L. The plasma concentration was maintained at this level during the continued infusion for 3 h. When the infusion was stopped, the plasma concentration declined at a similar rate in all experiments, the average t₁/₂ being 23.7-26.5 min. The same half-life, 26.8, was obtained in Exp. 4 when the infusion was terminated at 22.5 min.

In paper IV, data from 5 different studies (studies 1-5) were pooled in the pharmacodynamic analysis. In study 1, single oral (0.3, 0.6, 1.2, and 2.4 µmol/kg) and one intravenous dose of 0.3 µmol/kg of AR-H047108 were given to dogs (n=4). The plasma concentration time curves are shown in Figure 10. The plasma half-life of the metabolite was approximately 2.5 h, while the half-life of the parent compound was 1.2-1.4 hours.
Figure 10. Plasma concentration of AR-H047108 and AR-H047116 in the dog, after oral administration and after intravenous administration of AR-H047108 (Mean ± SE, n=4) (study 1 in paper IV).

In study 2, a single oral (3 μmol/kg) and single intravenous (0.5 μmol/kg) dose of AR-H047116 was given (n=2) (left graph of Figure 11). In study 3, a single oral (2.5 μmol/kg) and single intravenous (1 μmol/kg) dose of AR-H047116 was given (n=2) (right graph of Figure 11).
In study 4, AR-H047108 was given at a dose of 5 µmol/kg (n=4 in each experiment). The plasma concentration time curves (±SEM) of AR-H047108 and AR-H047116 are shown in Figure 12.

The mean plasma half-lives were 1.3 and 3.1 h for AR-H047108 and AR-H047116, respectively. The plasma concentration-time profile for each individual, in studies 1-4, was approximated by interpolation between time points.

In study 5, however, a model-based approach was used for the pharmacokinetic data. A one-compartment model with lag time was used to fit the plasma concentrations of metabolite following administration of metabolite at doses of 2.5 and 5 µmol/kg (PK1). A pharmacokinetic model was applied for the simultaneous fitting of drug and metabolite plasma concentration-time data following administration of drug at a dose of 2.5 µmol/kg (PK2), which took the formation of metabolite from parent...
compound into account. To be able to carry out all the acid secretion measurements on daytime, we had to dose at night on certain occasions. A covariate “night” was introduced in the pharmacokinetic models, because the plasma concentrations were low following administration at night compared to morning. The model predicted plasma concentrations were then used with interpolation in the pooled (studies 1-5) pharmacodynamic (PD) analysis. The pharmacokinetic parameters are presented in Table 2.

Table 2. Pharmacokinetic parameter estimates for AR-H047108 and AR-H047116 (data from paper IV).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PK1</th>
<th>PK2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population mean</td>
<td>Interindividual variability, %</td>
</tr>
<tr>
<td>$CL_{M/F}$ (L/min/kg)</td>
<td>0.33$^a$</td>
<td>34</td>
</tr>
<tr>
<td>$V_{M/F}$ (L/kg)</td>
<td>91</td>
<td>38</td>
</tr>
<tr>
<td>$Ka$ (min$^{-1}$)</td>
<td>0.16</td>
<td>130</td>
</tr>
<tr>
<td>$t_{lag}$ (min)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>$CL/F$ (L/min/kg)</td>
<td>0.21</td>
<td>40</td>
</tr>
<tr>
<td>$V/F$ (L/kg)</td>
<td>67</td>
<td>36</td>
</tr>
<tr>
<td>Residual Variability</td>
<td>13</td>
<td>25</td>
</tr>
</tbody>
</table>

$^a$CL$_{M/F}$ in PK1 means CL$_{M}$ over bioavailability.
$^b$CL$_{M/F}$ in PK2 is dependent on the degree of formation of metabolite.
5.2. Pharmacodynamics of irreversible proton pump inhibition

The PD data used in paper I show that low dose and short-term exposure to omeprazole result in a rapid recovery of acid secretion to baseline, whereas high-dose or extended exposure result in a rapid onset of inhibition and a slow recovery of response. A basic turnover model was applied to a combination of selected short-term and long-term experiments, but failed to describe the observed biphasic recovery of the response, as shown in Figure 13. A mechanistically based model for omeprazole action was developed, which could adequately describe the combined data set (Figure 13).

![Graph showing observed and model predicted response](image)

*Figure 13. Observed (●) and model predicted (lines) response for the basic turnover model (solid lines) and the mechanism-based model (dashed lines). A combination of selected short-term and long-term experiments in paper I was used to allow for discrimination between models. The left panel shows one representative long-term experiment (2.5 µmol/kg) and the right panel shows one of the short-term experiments (Exp 5=0.18 µmol/kg).*

The experiments in paper III were designed to provide suitable data to challenge the mechanism-based model. Four different experiments were carried out in each dog (n=4). For placebo and experiments 1-3, saline or omeprazole was infused for 3 h with measurement of histamine-stimulated gastric acid secretion in two periods of 3.5-6.5 hours, one period starting just before the omeprazole infusion and the later period up to 29 h post infusion. In experiment 4, omeprazole was infused for 22.5 min and gastric juice was collected for 5 h post infusion (Figure 14). The results of the experiments are compatible with the mechanism of action of omeprazole. A relatively rapid recovery of acid secretion was observed after the 22.5 min infusion (experiment 4). This may be due to the fact that only a fraction of the total amount of enzymes was inhibited, and that uninhibited enzymes was rapidly replenished from the pool.
The antisecretory effect increased during the 3 h omeprazole infusion (experiments 1-3), and then remained essentially constant over the remaining 2 h of histamine infusion. The rate of recovery was less rapid in these experiments, because a larger proportion of enzymes were inhibited. De novo synthesized enzymes will then be more important and determine the rate by which the gastric acid secretion returns to baseline.

Figure 14. Effect of omeprazole (◆ experiment 1, ■ experiment 2, ▲ experiment 3, ● experiment 4, ○ Control) on histamine-stimulated acid secretion in the Heidenhain pouch dog (Mean±SE, n=4). Grey bars indicate the histamine infusions of each experiment. Black bars indicate intravenous infusion of omeprazole, either for 3 hours (experiments 1, 2 and 3) or 22.5 min (experiment 4).

As the baseline response to histamine infusion can give further information about system specific parameters, the full profile of acid secretion, instead of deviation from baseline (which is the traditional way of treating the pharmacological effect of proton pump inhibitors), was modelled in this study. The model fitted the data well, with good agreement between observed and predicted acid secretion (Figure 15).
The obtained parameter estimates for the mechanism-based omeprazole model in paper I and III are shown in Table 3.

The half-life of turnover of enzyme, which determines the rate of recovery after extended exposure to omeprazole, $t_{1/2}(k_{out})$, was 54 hr in paper I and 17 hr in paper III. The half-life of the transfer of enzymes from active to resting state, $t_{1/2}(k_2)$, which determines the rate of recovery after low exposure, was similar in the two papers. In paper I, results from different study occasions were pooled and the routes of administration and activation of the baseline response on the different occasions were different. The second order rate constant for the irreversible binding of omeprazole, $k_{ome}$, was therefore allowed to be different and was estimated to 11 and 3 L/µmol/h for the intravenous and intraduodenal experiments, respectively. In paper III, $k_{ome}$ was estimated to 2.4 L/µmol/h. The potency, calculated as $k_{out}$ over $k_{ome}$, was in paper I 4.3 and 1.2 nM for the intraduodenal and intravenous doses, respectively and in paper III 16.7 nM. The first order rate constant for the transfer between P and R, $k_1$, was estimated to 0.4 h$^{-1}$ in paper I. In paper III, $k_1$ is second order and was estimated to 0.064 h$^{-1}$ per histamine concentration unit. A constant relating amount of active enzyme to the effect was estimated to 5 mmol H$^+$/30 min, which corresponds to the maximum acid secretion.

Additional analysis methods to nonlinear mixed effects modelling (referred to as “population”) were applied to fit the data in paper III (naïve pooling and standard two stage, referred to as “pooling” and “two-stage”, respectively). For all methods, similar parameter estimates, estimates of interindividual variability (only shown for “population”) and standard errors
(only shown for “population”) were obtained (Table 3). The observed and model-predicted response, obtained with the “population” method, is shown in figure 16.

Table 3. Omeprazole model parameter estimates (CV%) from papers I and III.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population</td>
<td>Pooling</td>
<td>2-stage</td>
<td></td>
<td></td>
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<tr>
<td>$k_1 (h^{-1})$</td>
<td>0.4</td>
<td>0.064</td>
<td>61</td>
<td>0.11</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(38)</td>
<td>(20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{1, his} (h/hist.conc.unit)$</td>
<td>2.0</td>
<td>1.9</td>
<td>62</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>(56)</td>
<td>(20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}(k_{out}) (h)$</td>
<td>54</td>
<td>17</td>
<td>65</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{ome1 (iv)} (l/µmol/h)$</td>
<td>11</td>
<td>2.4</td>
<td>63</td>
<td>1.4</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>(34)</td>
<td>(18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{ome2 (id)} (l/µmol/h)$</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(56)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Const (mmolH⁺)</td>
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<td>n.d.</td>
<td>4.1</td>
<td>6.3</td>
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<tr>
<td></td>
<td>(12)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Residual error (%)</td>
<td>20</td>
<td>34</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual error (mmolH⁺)</td>
<td>0.008</td>
<td>0.009</td>
<td>0.12</td>
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</tr>
</tbody>
</table>

In addition, predictions of acid secretion at various levels of histamine provocation and at the same omeprazole exposure were made. The predictions showed that the fraction of active enzyme is about 50 % during histamine infusion and that acid secretion and the amount of active enzyme decreases to zero when the histamine stimulation is turned off. This is expected since dogs have a basal acid secretion of <2 % of maximal acid secretion. When the omeprazole dose is kept constant but histamine stimulation is decreased, a more rapid recovery of response was predicted (Figure 17). Furthermore a slower increase and lower maximal response was predicted at lower histamine stimulation. This is consistent with the results of a previous study that showed that the inhibitory effect of omeprazole is dependent on the stimulatory state.45
Figure 16. Effect of omeprazole (◆ experiment 1, ■ experiment 2, ▲ experiment 3, ● experiment 4, ◊ Control) on histamine-stimulated acid secretion in the Heidenhain pouch dog. Grey bars indicate the histamine infusions of each experiment. Black bars indicate intravenous infusion of omeprazole, either for 3 hours (experiments 1, 2 and 3) or 22.5 min (experiment 4). The symbols are the observed values and lines are the individual fittings.

Figure 17. Predictions of acid secretion at various levels of histamine provocation at the same omeprazole exposure. The omeprazole doses used in the predictions were the doses given in experiments 4 (left graph) and 1 (right graph) (■ 100%, * 133%, ▲ 50%, ○ 33% and ● 10% of given histamine infusion in experiments 4 and 1, respectively).
5.3. Pharmacodynamics of reversible inhibitors

The effect-time profiles were somewhat delayed in comparison with the corresponding plasma concentration-time profiles for H 335/25 in dog (Figure 18). The maximum inhibition occurred 1-1.5 h following oral doses, whereas the peak plasma concentration was reached at 0.6-0.9 h. The inhibition then slowly declined with duration of inhibition that was longer than the observation period (5 and 4 h for dog and man, respectively). A similar effect delay was observed in man.

Figure 18. Plasma concentration (▲) and effect (●) versus time for one representative dog and dose. The maximum inhibition was delayed with respect to the plasma peak concentration for all doses.

The reason for this delay was elucidated by modelling the plasma concentration-effect relationship, using three different delay models; an effect compartment model, a turnover model and a binding model. A binding model best described the data, and the obtained parameters are shown in Table 4 for dog and man.

Predictions of the time course of the effect in man are shown in Figure 19 and appear to be robust towards the model chosen.
Table 4. H 335/25 model parameter estimates obtained in dog and man.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population</th>
<th>Intersubject</th>
<th>Population</th>
<th>Intersubject</th>
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</thead>
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<tr>
<td></td>
<td>mean</td>
<td>variability, %</td>
<td>mean</td>
<td>variability, %</td>
</tr>
<tr>
<td></td>
<td>(dog)</td>
<td>(dog)</td>
<td>(man)</td>
<td>(dog)</td>
</tr>
<tr>
<td>$k_{off} (h)$</td>
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<td>95</td>
<td>0.88</td>
<td>87</td>
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<tr>
<td>$k_{on} (L/nmol/h)$</td>
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<td>32</td>
<td>0.0035</td>
<td>Not estimated</td>
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<td>$K_d (nM)$</td>
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<td>250</td>
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</tr>
<tr>
<td><strong>Baseline</strong></td>
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</tr>
<tr>
<td>(mmol $H^+$/30 min)</td>
<td>2.03</td>
<td>0.9</td>
<td></td>
<td></td>
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<tr>
<td>(mmol $H^+$/15 min)</td>
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<td></td>
<td>8.02</td>
<td>15</td>
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<tr>
<td><strong>Residual</strong></td>
<td>0.18</td>
<td>0.74</td>
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<tr>
<td>(mmol $H^+$/30 min)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Figure 19. Prediction of the time course of drug action. The figure shows the individually predicted response for healthy study subjects following oral doses of 80, 150, 300, 500 and 800 mg with the effect compartment- (dashed lines), binding- (solid lines), and turnover model (dotted lines).
The inhibitory effect of AR-H047108 and its active metabolite on acid secretion was studied in paper IV. The maximum effect was delayed compared to the plasma concentration peak. A dose-dependent inhibition of the acid secretion was observed and the mean peak effect was reached earlier as the dose was increased (Figure 20). Histamine-stimulated acid secretion was measured in different periods after dose up to 24 h and data from different studies were pooled. An effect compartment and binding model, both in combination and separately were applied. When a possible secretion rate-dependent washout from the biophase was taken into account in the effect compartment model i.e. $k_{e0}$ was allowed to change with level of inhibition, the fit was improved compared to a constant $k_{e0}$. However, the combined model was shown to be the most appropriate model. The model parameters are shown in Table 5. The equilibration constant, $K_d$, for the parent compound was estimated to be 11 times lower than for the metabolite, indicating that the parent compound is more potent than the metabolite. The dissociation rate half-lives, $t_{1/2}(k_{off})$, for the metabolite and parent compound were 1.4 h and 3.8 h, respectively. The equilibration rate half-lives, $t_{1/2}(k_{e0})$, for the metabolite and parent compound were 1.5 h and 0.3 h, respectively.

![Figure 20. The mean acid secretion (± SEM, n=4) that was observed following different doses or vehicle (mean control) in study 1 of paper IV.](image-url)
Table 5. Population mean pharmacodynamic parameters (SE %) and associated intersubject variability (CV %) for drug and metabolite in dog obtained in the analysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Effect compartment (reduced model)</th>
<th>Binding compartment (reduced model)</th>
<th>Combined effect compartment and binding (full model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFV</td>
<td>-580</td>
<td>-699</td>
<td>-969</td>
</tr>
<tr>
<td>$k_{e0,\text{drug}} \ (\text{min}^{-1})$</td>
<td>$3.42\cdot10^{-3} \ (23)$</td>
<td>-</td>
<td>$3.51\cdot10^{-2} \ (23)$</td>
</tr>
<tr>
<td>$k_{e0,\text{met}} \ (\text{min}^{-1})$</td>
<td>$2.98\cdot10^{-3} \ (15)$</td>
<td>-</td>
<td>$7.49\cdot10^{-3} \ (26)$</td>
</tr>
<tr>
<td>IIV ($k_{e0}$)</td>
<td>$75b \ (55)$</td>
<td>-</td>
<td>$55b \ (46)$</td>
</tr>
<tr>
<td>$k_{\text{off,drug}} \ (\text{min}^{-1})$</td>
<td>-</td>
<td>$1.19\cdot10^{-3} \ (20)$</td>
<td>$3.03\cdot10^{-3} \ (22)$</td>
</tr>
<tr>
<td>$k_{\text{off,met}} \ (\text{min}^{-1})$</td>
<td>-</td>
<td>$1.36\cdot10^{-3} \ (21)$</td>
<td>$8.27\cdot10^{-3} \ (26)$</td>
</tr>
<tr>
<td>IIV ($k_{\text{off}}$)</td>
<td>-</td>
<td>$91b \ (55)$</td>
<td>$75b \ (27)$</td>
</tr>
<tr>
<td>$k_{\text{on,drug}} \ (\text{l/nmol-min})$</td>
<td>-</td>
<td>$1.39\cdot10^{-4} \ (15)$</td>
<td>$2.7\cdot10^{-4} \ (19)$</td>
</tr>
<tr>
<td>$k_{\text{on,met}} \ (\text{l/nmol-min})$</td>
<td>-</td>
<td>$1.74\cdot10^{-5} \ (16)$</td>
<td>$6.59\cdot10^{-5} \ (24)$</td>
</tr>
<tr>
<td>IIV ($IC_{50} \ or \ K_{d} \ (\text{nM})$</td>
<td>$81b \ (21)$</td>
<td>$39b \ (28)$</td>
<td>$46b \ (44)$</td>
</tr>
<tr>
<td>$IC_{50} \ or \ K_{d,\text{drug}} \ (\text{nM})$</td>
<td>$17.8 \ (28)$</td>
<td>$8.6^a$</td>
<td>$11.2^a$</td>
</tr>
<tr>
<td>$IC_{50} \ or \ K_{d,\text{met}} \ (\text{nM})$</td>
<td>$77.5 \ (18)$</td>
<td>$79.5^a$</td>
<td>$125^a$</td>
</tr>
<tr>
<td>Baseline (mmol H$^+$)</td>
<td>$2.83 \ (3.5)$</td>
<td>$2.84 \ (3.6)$</td>
<td>$2.69 \ (3.7)$</td>
</tr>
<tr>
<td>IIV (Baseline)</td>
<td>$24 \ (15)$</td>
<td>$24 \ (13)$</td>
<td>$25 \ (14)$</td>
</tr>
<tr>
<td>Residual variability (mmol H$^+$)</td>
<td>$0.307 \ (6.5)$</td>
<td>$0.28 \ (9.4)$</td>
<td>$0.212 \ (15)$</td>
</tr>
</tbody>
</table>

$^a$Calculated secondary parameter, $^b$the variability was assumed to be the same for drug and metabolite
6. Discussion

The inhibitory effect of gastric proton pump inhibitors is delayed in comparison to the plasma concentration-time profile. Despite the short plasma concentration half-life of about one hour for omeprazole, the duration of effect is several days after a single, standard dose. The duration of the reversible gastric acid secretion inhibitors is shorter, but still prolonged compared to the plasma concentration half-lives. In this thesis, PK/PD-modelling was used to better understand the underlying mechanism of the observed delays. This can help answer questions about the drug’s behavior, e.g., whether the rate of onset of effect will increase with a higher dose, how much longer the duration of action will be with a higher dose and the relative potency of a drug and its active metabolite *in vivo*.

A meaningful PK/PD relationship obtained in animals, has a potentially predictive value with regard to the situation in man. A prerequisite of this is a good pharmacological *in vivo* model, which gives information about potency, intrinsic efficacy and the time-course of the effect. Such information can be useful in preclinical decision making with regard to selection of drugs for further development. In addition, the time-course of response obtained in the animal model can be scaled to man through the allometric approach and therefore used to predict the first clinical dose. In hindsight, omeprazole is an example of why such characterization of pharmacodynamics is important in drug discovery, and why scaling of preclinical data should ideally include PD characteristics in addition to PK. In the case of omeprazole “poor” PK was saved by excellent PD properties.

In general, four processes are responsible for delays in the effect-time profile compared to the plasma concentration-time profile, namely distribution to the biophase, interaction with the receptor, indirect response or a rate limiting process in the chain of events after the receptor interaction leading to the effect. These processes can be described using mathematical models schematically illustrated in Figure 21. One plausible explanation for the delayed effect, of the reversible inhibitors studied, is that the high partitioning to the parietal cells could cause slow plasma biophase equilibrium, in which case an effect compartment model can be applied (Figure 21). However, the compounds studied are extensively bound to plasma proteins, which would shorten the time to plasma-tissue equilibrium.
In addition, if slow plasma-biophase equilibrium is the rate-limiting step, the time to maximum effect will be the same independent of dose providing linear kinetics. This was not the case. The peak effect was reached earlier as the dose was increased and this is to be expected if the delay is caused by a slow dissociation of the drug from the enzyme, in which case a binding model can be applied (Figure 21).  

Figure 21. Schematic illustration of four models for concentration-effect delays due to rate limitation in the interaction between drug and receptor and in pre- and post receptor events.
A rate limiting process in the chain of events after the receptor interaction leading to the effect can also cause an effect delay (Figure 21). A turnover model with inhibition of the production of effect was considered because the drug effect may be dependent on the acid present in the stomach when the drug is given. The time to maximum effect in the experimental data was found to decrease with increasing single doses of the reversible proton pump inhibitors. The turnover model, used for this particular data set, predicts the opposite; an increase in time to peak effect with increased dose. A transduction model was not applied because no additional steps between the drug-enzyme interaction and pharmacological effect are believed to exist for these compounds. Thus, depending on the model, higher doses may or may not alter rate of onset of effect. The delay between concentration and onset of drug effect, observed for the inhibitory effect of ranitidine on gastric pH in patients, was modelled by an indirect response model. Ranitidine was assumed to inhibit the production of gastric acid from the parietal cells. In the model, the observed tolerance development for this compound was taken into account. Usually, a combination of different processes is responsible for delays in biological effect, but it is often difficult to identify more than one responsible process because of the quality of data and/or counteracting mechanisms. This may indicate that further experimentation is needed. However, for AR-H047108 and its active metabolite, a model taking into account both slow plasma-biophase equilibration and prolonged binding to the enzyme was shown to be the most appropriate model. The obtained parameter estimates indicate, however, that slow dissociation of drug from the enzyme is the dominating process in the observed delay. However, the degree of prolonged binding varies between the compounds studied, which will result in different effect-time profiles for the compounds. For omeprazole, the dissociation is slow or nonexistent, whereas for the reversible inhibitors the half-life of the dissociation rate constant, $t_{1/2}(k_{off})$, ranged between 0.8-3.8 h, as shown in Table 6. The potency ($EC_{50}$) of the compounds differs as well, and this parameter will together with $t_{1/2}(k_{off})$ determine the duration of the effect of the reversible inhibitors.
Table 6. Comparison of obtained parameters for the compounds studied.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Omeprazole</th>
<th>H 335/25</th>
<th>AR-H047108</th>
<th>AR-H047116</th>
</tr>
</thead>
<tbody>
<tr>
<td>t_{1/2}(k_{off}) (h)</td>
<td>17 (^a)</td>
<td>0.8 (^b)</td>
<td>3.8 (^b)</td>
<td>1.5 (^b)</td>
</tr>
<tr>
<td>EC_{50} (\mu mol/L)</td>
<td>0.017</td>
<td>0.16</td>
<td>0.011</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\(^a\)enzyme turnover, \(^b\)release of drug from enzyme

In some cases, more complex forms of the basic turnover models may be needed. This will be the case if there is an asymmetry in the relationship between “on” and “off” responses as observed for omeprazole and for selective serotonin reuptake inhibitors (SSRI). For omeprazole, there is a slow or nonexistent dissociation of drug from the enzyme, and this has consequences for the effect-time profile. The standard delay models in Figure 21 cannot describe the case of biphasic recovery that was observed after omeprazole-induced inhibition of acid secretion in paper I and III. In contrast to omeprazole, the SSRI’s have a slow onset of effect and a rapid offset of effect. For these compounds it is not possible to predict the terminal phase of the effect from the acute phase. A physiologically based model for inhibition by omeprazole, lansoprazole and pantoprazole in rat and man was previously described. The model was built on the principle of reversible inhibition, taking into account that enzyme may be regenerated from the drug enzyme complex. Ferron et al., 2001, applied a basic turnover model for the irreversible, inhibitory effect of pantoprazole on gastric acid secretion in humans and rat. A stationary baseline was assumed for the acid secretion in man after pentagastrin stimulation, whereas in rat a decrease in acid output with time was taken into account. We developed a mechanism-based model for irreversible inhibition by omeprazole, in the dog, that separates drug-specific factors (pharmacokinetics and binding interaction) from system-specific factors (enzyme turnover and distribution). A basic turnover model was extended with a pool compartment, representing a reserve of inactive enzymes that becomes partially activated upon stimulation of acid secretion. Elimination of enzyme takes place from both compartments, and the half-life of the fractional turnover rate of the enzyme, t_{1/2}(k_{out}), was estimated to 17 hours in the dog (paper III). By using a mechanism-based model, the effect of different stimulatory states of acid secretion on the inhibitory effect of omeprazole could be studied. In paper III, the full acid secretion profile was used, that is, the histamine-induced
baseline response was modelled simultaneously with active treatment. With this approach further information about the system properties could be obtained. Predictions of the response could then be made following different exposures of omeprazole and different histamine provocations, which could be useful in the design of future experiments.

In this thesis, the chain \textit{plasma concentration-enzyme inhibition-acid output} was modelled. Acid output is, however, not easily correlated to clinical effect. Modelling of the link between plasma concentration and intragastric pH would be useful, because intragastric pH has been shown to correlate with symptoms, such as heartburn, and esophageal damage.\textsuperscript{15} A PK/PD-model for lansoprazole that could describe the full intragastric pH-time profile over 24 hours, and also taking the circadian rhythm of acid secretion and food effect on intragastric pH into account was described by Puchalski \textit{et al.}, 2001.\textsuperscript{59} Such a model could be useful in study design, prediction of optimal dosing regimens and in the study of differences in effect between different patient populations.
7. Concluding remarks

In paper I, a mechanistically based PK/PD-model was developed. Data from different preclinical studies were pooled and used in the analysis. The model could adequately describe how the duration of the effect of omeprazole is determined by the exposure of drug in relation to the amount of activated enzymes. In histamine-stimulated dogs, a low exposure to drug resulted in a rapid recovery of acid secretion. This was probably due to the fact that only a fraction of the total amount of enzyme was inhibited and that uninhibited enzyme was recruited rapidly from the tubular membranes of the parietal cells. The rate of recovery was less rapid following higher exposure. This may be due to a larger proportion of the total amount of enzymes being inhibited because omeprazole did not only decrease the number of enzymes that was activated at drug administration, but also inactive enzymes from the tubular membranes that eventually became activated. De novo synthesized enzymes would then be more important for the recovery rate of acid secretion. The model predicted that at the applied stimulation level of acid secretion in the dog only about 20 % of the total number of enzymes were activated.

The aim of paper II was to describe the PK/PD-relationship of a reversible gastric acid proton pump inhibitor. In contrast to the irreversible inhibitors, for reversible inhibitors the pharmacokinetics will be the major factor determining the effect-time profile. However, the effect-time profile is delayed compared to the plasma concentration-time profile. Acid output data, calculated as deviation from histamine-stimulated baseline values, from dose escalation studies in dog and man were used. It was shown that there was a delay in the onset of the effect and that the duration of the effect was prolonged compared to what could be expected from the plasma concentration-time course. Possible explanations for this delay may be a rate limiting binding interaction between enzyme and drug and/or accumulation of drug within the acidic space of the parietal cells because of their weak base properties. Different standard delay models were tested in order to quantify and understand the mechanism of the effect on acid secretion. A kinetic binding model was shown to function the best. With a binding model it is assumed that the time course of the effect is determined by the interaction between enzyme and drug and not by the turnover of enzyme, as
in the case of omeprazole. According to the binding model, the delay may be
explained by a slow dissociation of the drug from the enzyme.

In paper III, new experiments were conducted in order to challenge the
proposed model in paper I. Another aim was to characterize the influence of
histamine stimulation on acid secretion. The experiments in the study were
designed to obtain suitable response data both early and at later times after
omeprazole dosing. The model was found to describe the PK/PD-
relationship established in paper I adequately. The full profile of the acid
secretion was modelled in this study instead of deviation from a histamine
stimulated baseline value, which is the traditional way of calculating the
effect variable in these studies. By modelling the full profile, predictions of
the effect on acid secretion are possible not only following different
exposures of omeprazole, but also following different histamine
provocations. Predictions of the effect of histamine on acid secretion, when
the omeprazole dose was unchanged, showed that a lower histamine
stimulation results in a more rapid recovery of acid secretion. In addition, a
slower increase in, and a lower maximal acid secretion is obtained when the
histamine stimulation is decreased. Varying drug exposure and histamine
provocation simultaneously may be a more optimal way of obtaining
information about system parameters and this may be useful in the design of
new studies.

In paper IV, another reversible inhibitor with an active metabolite was
studied. The aim was to describe the PK/PD-relationship and determine the
relative potency of the drug and its metabolite. A combined effect
compartment and binding model was shown to best describe the relationship.
It was assumed that the metabolite bind to the same enzyme, H⁺, K⁺-ATPase
as the parent compound. Thus, the combined effect of the parent drug and
metabolite on acid secretion was modelled as a competitive interaction
between the compounds. The metabolite was found to be 1/3 as potent as the
parent compound in vitro, but it is generated pre-systemically to a large
extent and rapidly reaches higher concentrations than the parent compound,
in the dog. Considering this and also the longer plasma concentration half-
life of the metabolite, the relative contribution of the metabolite could be
expected to increase with time after dose and prolong the duration of action
despite its lower potency. The model predicted that the metabolite was less
potent than the parent compound in vivo. For both compound the
dissociation from the enzyme is rate limiting. In addition, the high
partitioning of the compounds into the canaliculus of the parietal cells
appears to contribute to the delay.
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My daughter Amelia, for being. ♥

Angela
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