

Review article: the control of gastric acid and *Helicobacter pylori* eradication

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Accepted for publication 5 June 2000

SUMMARY

This review focuses on the gastric acid pump as a therapeutic target for the control of acid secretion in peptic ulcer and gastro-oesophageal reflux disease. The mechanism of the proton pump inhibitors is discussed as well as their clinical use. The biology of *Helicobacter pylori* as a gastric denizen is then discussed, with special regard to its mechanisms of acid resistance. Here the properties of the products of the urease gene clusters, ureA, B and

ureI, E, F, G and H are explored in order to explain the unique location of this pathogen. The dominant requirement for acid resistance is the presence of a proton gated urea transporter, UreI, which increases access of gastric juice urea to the intrabacterial urease 300-fold. This enables rapid and continuous buffering of the bacterial periplasm to \approx pH 6.0, allowing acid resistance and growth at acidic pH in the presence of 1 mM urea. A hypothesis for the basis of combination therapy for eradication is also presented.

INTRODUCTION

In the last 25 years of the 20th century, a series of revolutions occurred in our understanding of the biology of gastric acid secretion and of acid related disease, particularly with respect to the role of *Helicobacter pylori*. Whereas 'no acid, no ulcer' remains as true as it were in 1910, a new aphorism is required to state our current therapeutic strategy. At the beginning of the 21st century, the treatment and outcome of acid related disease have changed radically. Peculiarly, these changes, although remarkable, have passed largely unheralded by the general public. On the other hand, we have become much more aware of the long-term consequences of gastro-oesophageal reflux disease

(GERD) and of *H. pylori* infection, consequences that will again change our method of treatment.

The introduction of histamine-2 receptor antagonists allowed, for the first time, successful, relatively routine, medical management of peptic ulcer disease.¹ However, it then became clear that duodenal ulcer treatment required maintenance therapy with these drugs, raising the question as to the reasons for recurrence.² Additionally, the effectiveness of these antagonists against not only histamine-, but also gastrin-stimulated secretion, placed the release of histamine as the pivotal event in the regulation of acid release from the parietal cell.¹ This, in turn, led to the recognition of the enterochromaffin-like cell rather than the mast cell, as the master neuro-endocrine cell regulating parietal cell function.³

After their introduction, it was realized that the clinical effectiveness of these receptor antagonist drugs was somewhat limited, even at high doses. Whereas acute treatment and maintenance of duodenal ulcer disease was satisfactory, certainly compared to before their

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introduction, the treatment of gastro-oesophageal erosion was less so. However, at the same time as the first of these receptor antagonists was introduced, the acid pump of the stomach (the gastric H, K ATPase) was described and its potential as a better therapeutic target was recognized.⁴⁻⁶

The drugs that were introduced as an improvement in therapy, particularly of GERD, were targeted at the acid pump. Now, truly reliable treatment of all types of acid related diseases became available in the form of proton pump inhibitors.⁷ However, the problem of recurrence of peptic ulcers was still present in spite of superior inhibition of acid secretion during initial therapy.

At the same time that the first proton pump inhibitor (omeprazole) was patented (1979), a pathologist in Western Australia, Robyn Warren, was intrigued by the observation that bacteria were present at the sites of ulcer disease. He managed to convince a young resident, Barry Marshall, that this might provide an explanation for ulcer recurrence and the door to *Campylobacter* now *Helicobacter pylori* was opened.⁸ Today it is recognized that infection by this organism is the major culprit in peptic ulcer disease and eradication is now commonly performed by a combination of a proton pump inhibitor and amoxycillin and clarithromycin.⁹ This review will discuss the last two of these advances, the proton pump inhibitors and their mechanisms and profiles and the peculiarities of an organism that has chosen the stomach as its favoured habitat.

THERAPEUTIC ASPECTS OF THE GASTRIC H, K ATPASE, THE GASTRIC ACID PUMP

Ion transport cycle of the acid pump

The gastric H, K ATPase is an ion-motive ATPase, belonging to a family of these, called the P type ATPases. These ATPases transport ions as a function of a cycle of phosphorylation and dephosphorylation of the transport protein.¹⁰ A subfamily of these P type ATPases are the P₂ ATPases, which transport small cations such as Na⁺, K⁺, Ca²⁺, Mg²⁺ and H⁺. In this P₂ group are the closely related Na, K and H, K ATPases distinguished by the presence of two, rather than one, sub-units, a catalytic, ion transporting sub-unit (the alpha sub-unit), and a stabilizing sub-unit (the beta sub-unit).¹¹ The Na, K and H, K ATPase are about 65% homologous. However, digoxin is absolutely selective

for the Na, K ATPase whereas the proton pump inhibitors, owing to their chemical properties, are absolutely selective for the gastric H, K ATPase. These ATPases have six membrane inserted segments which are regarded as their core structure containing the ion transport pathway and either two such segments preceding the core structure to give eight membrane segments in the transition metal pumps (P₁ ATPases) or four segments following the core structure to provide 10 segments in the P₂ ATPases. The beta sub-unit has a single trans-membrane segment.

The gastric pump is present in high concentrations in the parietal cell and at much lower concentrations in the collecting duct of the kidney. Whereas maximal acidity in the parietal cell canaliculus is <1.0, that in the kidney is never less than 4.0. Whatever the pathway of stimulation of the parietal cell, the common event is recruitment of the pump, present in cytoplasmic tubules into the membrane of the secretory canaliculus, to form microvilli lining this space, and activation of a KCl pathway to allow K⁺ to access the external surface of the ATPase and secretion of Cl⁻.

The coupling between the cycle of phosphorylation and dephosphorylation and ion transport is best described as due to a sequential set of conformational changes. In the absence of MgATP the pump is in a conformation able to bind MgATP and the outward ion (H₃O⁺), the E₁ conformation. With the binding of MgATP and the ion, the protein is phosphorylated in a sequence common to all the P type ATPases and the ion moves into the membrane domain of the catalytic sub-unit (E₁-P). Within the membrane domain, the transport ion is enclosed so that it loses communication with the cytoplasmic side and has not yet opened to the outside face, the occluded conformation. The outside face now opens forming the E₂-P conformation. At this stage the outward ion is extruded and the inward ion binds. With this, the pump dephosphorylates (E₂ conformation), followed by the inward ion, become occluded. From this state, the ion-binding site opens to the interior and the inward ion is released as MgATP rebinds. Figure 1 illustrates these steps for the gastric H, K ATPase.

The pump is thought to pump hydronium ions (H₃O⁺) rather than protons, since at high pH values it is able to pump Na⁺. It is able to release H₃O⁺ at a concentration of 160 mM, therefore generating an external pH of 0.8. This corresponds to a 4 million-fold gradient with respect to the inside of the parietal cell. It is able to

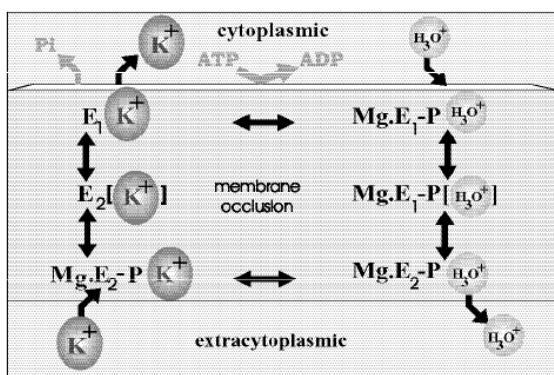


Figure 1. The reaction cycle of the gastric H^+ , K^+ ATPase showing the E_1 form of the enzyme that is phosphorylated by ATP as the hydronium ion, H_3O^+ , is bound. This form converts to $\text{E}_2\text{-P}$ as the hydronium ion is released after passing through the trapped or occluded form. With binding of K^+ , the cycle progresses by dephosphorylation, occlusion and release of K^+ to the cytoplasm from the E_1 form of the enzyme.

reabsorb K^+ , generating an inward gradient of about tenfold. As such, this is the most powerful pump known in mammalian systems and generates an acidic space within the secretory canaliculus of the parietal cell at least a thousand-fold greater than anywhere else in the body. In the absence of K^+ on its external surface, the pump locks into the $\text{E}_2\text{-P}$ conformation and does not turnover. Essential for activation of acid secretion is the presentation of K^+ to the outside surface of the pump. This occurs as the pump moves into the microvilli of the secretory canaliculus due to activation of a K^+Cl^- pathway.

Structure of the ATPase

The three-dimensional structure of these pumps is becoming clearer so that these conformational changes can be described in molecular terms. There are four distinct domains of the catalytic sub-unit: a large cytoplasmic domain, a stalk domain which connects the cytoplasmic to the membrane domain, and an extracytoplasmic domain. The cytoplasmic domain undergoes large conformational changes as the ion is transported outwards, changing from a loose structure to a more compact one as the ion moves to the external surface (E_1 to E_2 transition). The ion passes through the stalk and into the membrane domain, which also changes conformation as the ion binding site changes

orientation from facing inward to facing outward. The cytoplasmic domain in the ATP and inward ion binding conformation (E_1) is a relatively open structure with at least two large separate lobes, corresponding to the loops between M2 and M3 and M4 and M5. The latter contains the phosphorylation site and MgATP binding region. In the ion site facing outward ($\text{E}_2\text{-P}$) conformation, the cytoplasmic domain is more compact and the separation between the lobes less evident.

The stalk contains the extensions of the membrane segments into the cytoplasmic region and forms a link for the passage of ions into the membrane. The membrane domain of the catalytic sub-unit of the H, K ATPase contains 10 inserted segments. The arrangement of these segments and the relative mobility of these is vital for the transport of the ions. The beta sub-unit has a single transmembrane segment with most of the protein on the outside surface of the pump, closely associated with the beginning of M8 in the catalytic sub-unit. It is N-glycosylated at seven sites. Its function appears to be stabilization of the membrane region of the alpha sub-unit, perhaps to lock TM8 in place so that K^+ can bind for inward transport. The external domain contains the loops connecting the membrane segments of the catalytic sub-unit and most of the beta sub-unit, with six or seven N-linked glycosylation sites. The largest loop between the membrane segments of the alpha sub-unit is between M7 and M8.

Different biochemical and mutagenesis experiments have provided evidence that there is a close association between M4 and M6, M5 and M7 and M6 and M9. Recent data on 2D and three dimensional crystals of the sarcoplasmic reticulum (sr) Ca^{2+} ATPase have allowed placement of the membrane segments relative to each other and soon we should be able to place the side-chains of the membrane-inserted segments in their appropriate positions to reveal the ion pathways through the membrane. Prior to crystallization a series of site directed mutations have been made, which implicate M4, M5 and M6 as being intimately involved in the pathway transporting H_3O^+ outward and K^+ inward.

Activation of the H, K ATPase

In the absence of stimulation, the vast majority of the pump molecules present in the parietal cell are in cytoplasmic membrane structures called tubulovesicles. Upon stimulation, these tubulovesicles transform into

the microvilli of an infolding of the parietal cell, termed the secretory canaliculus. Along with this transformation, the canaliculus membrane acquires the property of a KCl conductance, thus supplying K^+ to the outside surface of the enzyme. This enables the enzyme to cycle, exporting H^+ and reabsorbing K^+ , leaving the Cl^- to accompany the secreted H^+ . Various proteins, such as ezrin and Rab, generally involved in membrane recycling, have been identified in this activation mechanism. In humans, there is continuous recycling of the pump between the tubulovesicles and the secretory canaliculus, to give a basal acid secretion. Upon stimulation by food, there is a rapid recruitment of the pump into the canaliculus membrane.

Towards a three-dimensional map of the H, K ATPase

Recently, three dimensional reconstructions have been published of the H^+ ATPase of *Neurospora* and the Ca^{2+} ATPase of the sarcoplasmic reticulum.^{12, 13} These

confirm the presence of 10 membrane segments in the catalytic sub-unit of the P_2 type ATPases and show the likely presence of a vestibule at the luminal face of the enzymes. They both show a large cytoplasmic sector, a stalk and a membrane sector with a small external sector. The advent of three dimensional crystals of the sr Ca^{2+} ATPase at 3.7 Å resolution (Toyoshima, C) will do much to advance the field. The model shown in Figure 2 illustrates the different associations by other methods that have been described in the Na, K, Ca and H, K ATPases, where M1, M2 and M3, M4 are juxtaposed and M4, M5, M6 and M8 are close to the transport pathway of the pump and M9, M10 and the beta sub-unit are peripheral to the transport sector.

Putative transport mechanism

The rate of ion transport by a P_2 type ATPase is about 10^4 times slower than through a channel and 10^2 times

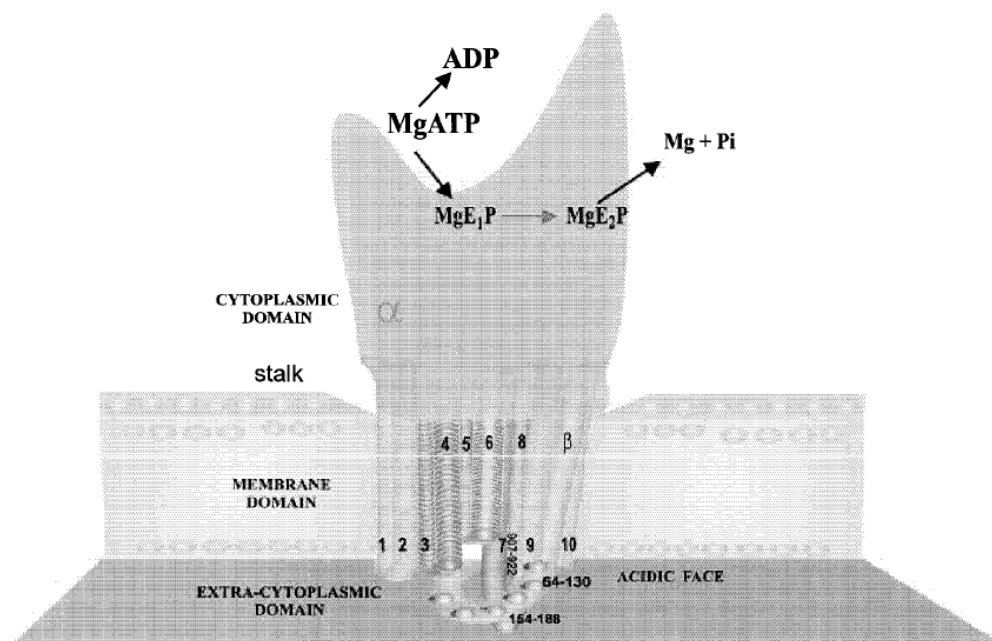


Figure 2. The probable arrangement of the membrane segments of the alpha and beta sub-units of the H^+ , K^+ ATPase with interactions of M8 and beta residue regions numbered and M6 to M9 and M5 to M7 numbered at the end of M8 in the figure. The transport region of the membrane domain is considered to involve particularly M5 and M6 along with M4 and perhaps M8, as illustrated schematically. The cytoplasmic domain is visualized with a groove-admitting ATP and ion. The groove closes as the enzyme goes from E_1 to E_2 . The majority of the beta sub-unit is on the outside surface and in the case of hog enzyme has 6 N-linked glycosylation sites. A vestibule is present bounded by M4, M5, M5-M6 loop M6 and M7, M8.

slower than through a carrier. This is due to the larger conformational changes necessary in a pump, compared to these other types of transporters. Several approaches have shown that these ATPases undergo conformational changes as a function of ion binding. For example, the fluorescence of FITC labelled H, K ATPase is rapidly quenched with the addition of K^+ as is the Na, K ATPase.^{14, 15} The rate of exchange of tritium or deuterium is markedly altered as the E_1 conformation changes to E_2 .^{16, 17} Electron diffraction images of the pumps in the E_1 or E_2 state show large changes in shape of the cytoplasmic domain.^{18, 19}

The location of ion transport is through the membrane domain. In the case of the Na, K, Ca and H, K ATPases, the ion transport pathway becomes occluded during the transport cycle, that is to say the ions are trapped within the membrane domain as the pump transits from E_1 to E_2 and vice versa, as if a gate on either side of the ion is closed during ion movement across the membrane domain. Site directed mutagenesis has identified several residues within the membrane domain as relevant to occlusion and transport. In particular, hydrophilic amino acids such as aspartic acid, glutamic acid, serine and threonine in M4, M5, M6 and perhaps M8 have been identified by this approach as related to ion flux through the membrane. M5 and M6 have also been identified as probably mobile within the membrane domain, as a function of loss of K binding after trypsinization.^{20, 21}

The crystal structure of other ATPases of the same family suggests the presence of a luminal vestibule in the pump. By analogy to the structure of the K channel of bacteria that has been resolved, the vestibule contains binding sites for K^+ that remove the water of hydration from the ion as it moves through an inverted filter funnel and then moves through the transport site as the naked ion. In the K channel, this was recognized even before crystallization since hydrophobic amines blocked K movement. In the case of the H, K ATPase a series of hydrophobic amines, such as imidazopyridines, quinazolines or aza-indoles are all K^+ competitive inhibitors binding at the luminal surface.^{22–24} Site directed mutations have identified residues in M3/M4 and M5/M6 as involved in binding an imidazo-pyridine, SCH28080. These may then be considered to form part of the vestibule admitting K^+ .^{25, 26}

If so, then the region containing M5 and M6 and the connecting loop between these membrane segments is a target for hydrophobic drugs able to penetrate the

luminal vestibule of the pump. An example of this class of drug is the proton pump inhibitors.

THE PROTON PUMP INHIBITORS

Mechanism of the proton pump inhibitors

All the proton pump inhibitors have a similar core structure, a substituted pyridyl methylsulfinyl benzimidazole. The pyridine moiety gives them the property of being a protonatable weak base with pK_a in the range of 4.0–5.0. In the un-protonated form they are membrane permeable prodrugs, so that they will concentrate in spaces more acidic than pH 4.0. In people, the only space where the pH can go lower than 4.0 is the canaliculus of the active gastric parietal cell. They are prodrugs since these compounds are subject to a H^+ catalysed conversion where they undergo a rearrangement to sulfenic acids and thence to sulfenamides.²⁷

The latter are the product in acidic solutions but the former may be responsible for inhibition of the ATPase under acid secreting conditions. As a class, these drugs are therefore acid-unstable, requiring protection against gastric acidity for oral formulation and requiring reconstitution for i.v. administration. The four compounds available differ in terms of acid stability. Pantoprazole is the most stable, followed by omeprazole and lansoprazole and then rabeprazole. The first three have a pK_a of ≈ 4.0 whereas the pK_a of rabeprazole is close to 5.0. The higher pK_a of rabeprazole accounts for its relative acid instability but this drug will also concentrate 10-fold relative to the other compounds, hence it will concentrate 10-fold in an acidic space of pH 4.0, compared to no accumulation for the other drugs. At the pH of a fully activated parietal cell canaliculus, accumulation is theoretically 1000-fold for omeprazole, lansoprazole and pantoprazole and 10 000-fold for rabeprazole.

The acid space accumulation is a key feature of these drugs, giving them a large therapeutic index, since they are highly concentrated at their target, the site of acid secretion. Following this, their conversion to a reactive sulfenic acid or sulfenamide (which form disulphides with one or more of the accessible cysteines in the pump) provides an ability to covalently inhibit the gastric ATPase, providing acid inhibition of long duration even after the drug has disappeared from the blood. Both the sulfenic acid and the sulfenamide are permanent cations, and are therefore relatively

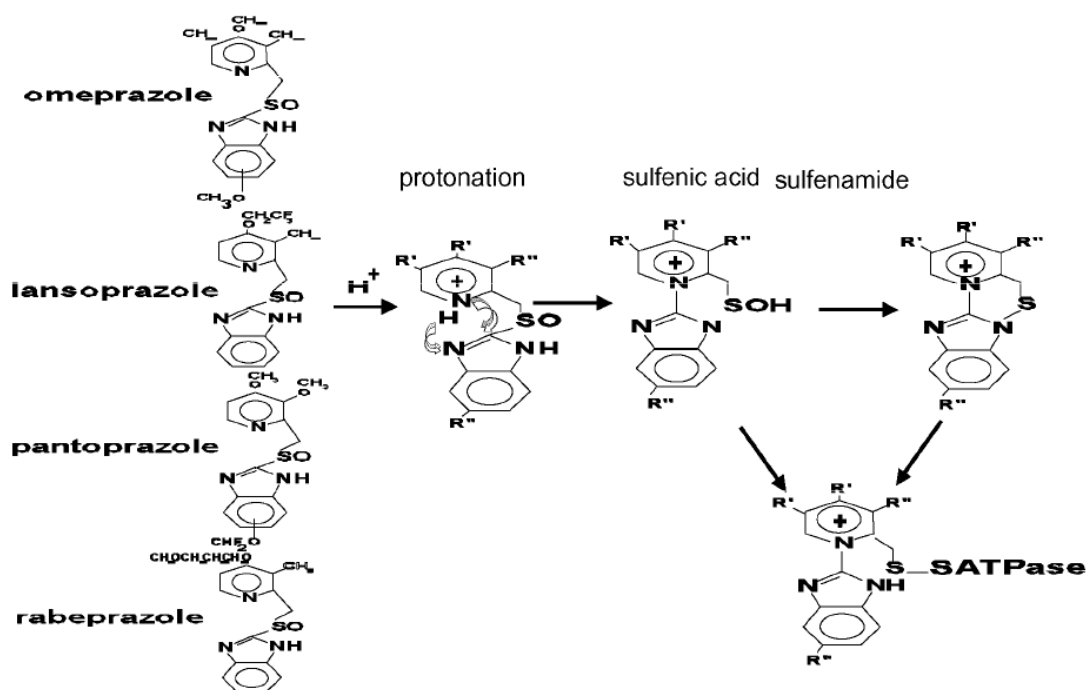


Figure 3. The chemistry of proton pump inhibitors. The four currently available drugs are shown on the left. The protonation step results in selective accumulation in the secretory canaliculus of the parietal cell. In acid, there is an acid catalysed conversion to the sulfenic acid and thence to the sulfenamide. Either of these can inhibit the H, K ATPase although it appears more likely that the sulfenic acid is the primary inhibitor.

membrane impermeant, remaining trapped at their site of formation or being secreted into the gastric juice. Their mechanism of action is illustrated in two figures, one detailing the putative chemical mechanism (Figure 3), the other superimposing their mechanism onto the illustration of the pump to define their target pictorially (Figure 4).

Clinical effects of proton pump inhibitors

Kinetics of inhibition. The drugs have become the mainstay of therapy for GERD and are also used in the treatment of duodenal and gastric ulcer disease, especially since treatment of many of these patients involves eradication of *H. pylori*.

Since proton pump inhibitors are covalent and require acid secretion for maximal effect, their use requires a degree of understanding of these characteristics for rational treatment of patients. The drugs are given in association with food, so as to stimulate the parietal cell to make acid. They are also protected to varying extents,

based on their formulation, from gastric acidity. They all have a relatively short plasma half-life. They therefore appear in the blood about 30 min after oral dosage and are present in the blood for about 90 min. Therefore, the window of opportunity for inhibition of active gastric pumps is about 90 min. After 90 min, if there are pumps that were inactive but now can be activated, they will not be inhibited since the prodrug has disappeared from the blood. It can be estimated, from the rate of onset of inhibition by proton pump inhibitors, that about 75% of the pumps are morning meal activated.

On the other hand, those pumps that are inhibited are inhibited covalently and thus inhibition of these pumps lasts well beyond the plasma dwell time of the compounds. Two possibilities exist for these inhibited pumps: either the inhibition is not reversed and eventually the pumps are consumed by the normal protein degradative pathways, or there is reversal of inhibition by glutathione as a reagent able to break the disulphide bond and reverse the inhibition. In contrast, there is continuing *de novo* synthesis of pumps; in the

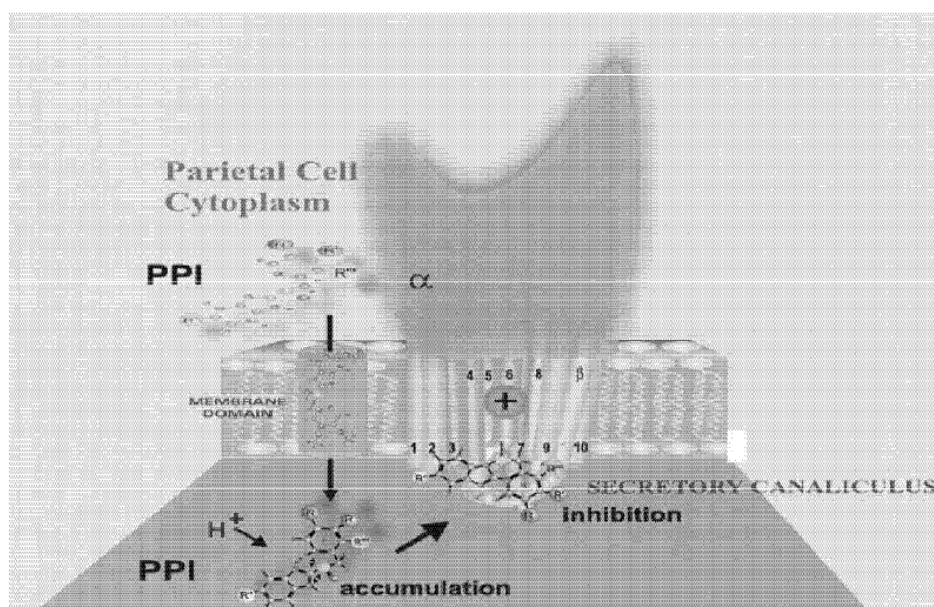


Figure 4. A pictorial representation of the mechanism of inhibition of the gastric proton pump by proton pump inhibitors. Illustrated is the drug in the cytoplasm of the parietal cell diffusing through the membrane of the canaliculus and accumulating due to protonation. The sulfenic acid then forms a disulphide with cys 813 in the loop between M5 and M6, to form a covalently inhibited complex. Other cysteines can also react but may not be directly involved in inhibition.

rat, the half-life of the pump protein is ≈ 50 h.²⁸ This means that about 25% of pumps are replaced each day. If then, there is no reversal of the disulphide bond, it is predicted that steady state inhibition would, on once-a-day morning dosage, happen on the 3rd day of treatment and would be about 66% of stimulated acid output. For example, on the first dose, 25% of pumps would remain and by the next morning 50% of the normal complement of pumps would be present due to replacement by *de novo* synthesis. With this dose, 75% of these would be inhibited, leaving only 12.5% of the normal complement of pumps present immediately after dose. By the next morning, 37.5% of the normal secretory capacity would be present and after a further dose of the drug, again 75% active, 9% of capacity would remain immediately after the dose. On the third morning, after synthesis of again, 25% of pump protein, only 34% of secretory capacity would remain. With further iteration of this calculation, no significant increase in acid inhibition would occur. In order to improve the rate of onset of inhibition, either a very large dose can be given so that the drug can remain at high levels in blood for longer than 90 min, or divided

doses are given along with meals either once or twice a day. On twice-a-day dosage, inhibition of acid secretory capacity improves to about 80% of maximally stimulated output. Although there are claims of a more rapid onset of inhibition with some of the drugs available, there are as yet no clinical data establishing that these claims provide superior results.

Just after these drugs were introduced, it was feared that their use would result in achlorhydria and consequent bacterial overgrowth in the stomach. The idea was that these were irreversible inhibitors of the gastric ATPase and that this translated into irreversible inhibition of gastric acid secretion. This is clearly not the case, as illustrated by the arithmetical argument above, and achlorhydria is not found with treatment using any oral proton pump inhibitor.

Therapeutic target for pH elevation. A number of clinical trials have established the superiority of proton pump inhibitors, compared to H_2 -receptor antagonists, for the treatment of any acid related disease.

However, the margin of superiority varies according to the success of the receptor antagonist for a given

disease. In the case of healing of GERD, moderately severe to severe, proton pump inhibitors are effective to about the 90th percentile within 4 weeks, compared to the 65th percentile for receptor antagonists. They are also significantly better in symptom relief and prevention of heartburn.

Additionally, there is superiority in treatment of duodenal and gastric ulcers, whether NSAID related or *H. pylori* related. In earlier trials, infection was not considered and overall, proton pump inhibitors healed peptic ulcers twice as fast as H₂-receptor antagonists.

In a series of meta-analyses of more than 300 clinical trials where the degree of pH elevation was plotted against the rate of healing, the optimal pH for healing of GERD and duodenal ulcers was estimated, based on predicted daily median pH achieved by H₂-receptor antagonism and by omeprazole as a representative proton pump inhibitor. It appears that achieving a median pH of 3.0 for 18 h per day is sufficient for 'optimal' healing of duodenal ulcer disease.²⁹ This optimum is deduced from the pH expected from current proton pump inhibitor treatment. In the case of GERD, a median pH of 4.0 is required for optimization.³⁰ Re-plotting the values obtained from these meta-analyses allows a direct comparison of proton pump inhibitors with H₂-RAs as therapeutic contenders for healing of duodenal ulcer and GERD, as shown in Figure 5. A discussion of the use of these agents in the

eradication of *H. pylori* will be discussed in the second segment of this review.

PROTON PUMP INHIBITORS AS DRUGS

There are four proton pump inhibitors currently on the market: omeprazole, lansoprazole, pantoprazole and rabeprazole, the most recent of the proton pump inhibitors. In this section we shall discuss their use mainly for GERD and purely NSAID-induced gastric ulcers. Their employment in the eradication of *H. pylori* will be dealt with in a separate section of this review.

GERD

Gastro-oesophageal reflux disease is a rapidly growing ailment world-wide. Although the acid (or peptic) damage to the oesophageal epithelium is due to an incompetence of the lower oesophageal sphincter, effective therapy directed at normalizing its complex relaxation pattern has not surfaced. Hence acid control is the effective method of choice for treatment of simple heartburn to grade IV erosions.

A guiding postulate of treatment for healing of GERD based on meta-analysis is, as shown in Figure 5, to elevate median intragastric pH to at least 4.0 for 18 h or more per day. This is predicted to optimize healing

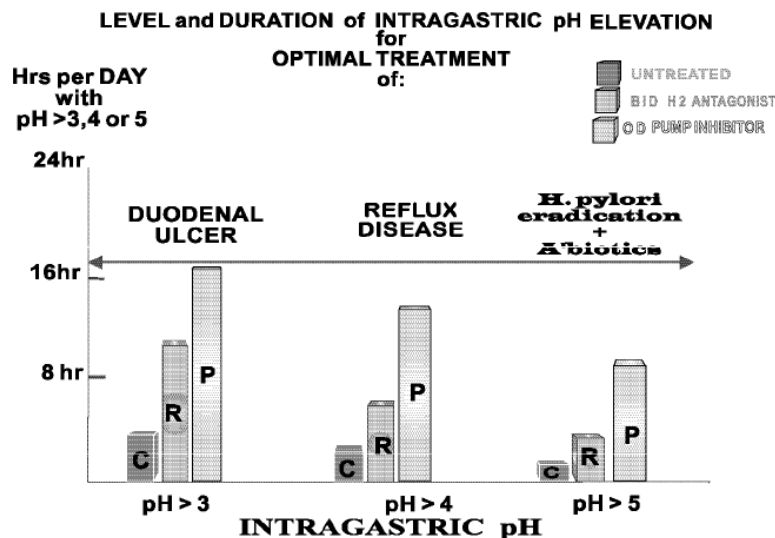


Figure 5. Meta-analysis of the pH elevation and duration of elevation required for optimization of healing of duodenal ulcer and GERD (modified from^{29, 30}). C, untreated; R b.d., ranitidine; P., omeprazole o.d.

and has, perhaps, a rational basis. The disease is caused by the incompetence of the lower oesophageal sphincter, allowing acid and other gastric contents to reflux into the oesophagus. Although the major damaging agent is acid, as evidenced by the efficacy of acid secretory inhibition, other factors such as pepsin and perhaps bile may also play a role in determining the severity of the disease. In the absence of effective and safe medical anti-reflux therapy, effective acid suppression is at this time the only medical option.

With the increasing incidence of oesophageal cancer related to the effect of acid on the oesophageal epithelium, resulting in the metaplasia of Barrett's disease, a pre-cancerous state, treatment of acid reflux is becoming more heroic, not only attempting to heal the lesions induced by reflux, but also aiming for rigid control of symptoms. Hence it is not only control of acidity to a mean diurnal pH of >4.0 but prevention of any excursions to a pH < 2.0 that may be desirable targets. Here, even large doses of H₂-RAs (q.d.s.) do not achieve the pH of 4.0 required for optimal healing, whereas once-a-day dosage of proton pump inhibitors is usually effective. Medical treatment of GERD therefore requires proton pump inhibitors as first line therapy since other agents are less effective for acid suppression.

The human oesophageal epithelium is a multilayered, stratified squamous epithelium. Afferent nerves are present reaching into the superficial layers of the epithelium. Rather than being protected by a continuous tight junction, this epithelium has largely regional cell-to-cell contact via desmosomes. The multilayered nature of the epithelium provides a winding path for proton diffusion into its deeper layers. It is significantly more acid sensitive than the gastric epithelium that is provided with 'tight' junctions.

The threshold of acidity for damage and pain is probably not the same. A greater acidity is probably needed for damage to the epithelial cells compared to the acidity required for stimulation of the pain fibres. This is because the epithelial cells have acid recovery mechanisms (such as Na⁺/H⁺ or anion exchange) not present in the pain fibres. Dependent on the location of the pain fibres and access thereto of H⁺, individuals will have different sensitivities to luminal acidity.

Given the absence of continuous tight junctions, or at best leaky tight junctions, the lower oesophageal epithelium is an inviting target for acid-back diffusion. Mucus, being an aqueous gel, is unlikely to provide a significant barrier to acid diffusion. Resistance to acid

depends more on the ability of the oesophageal epithelium to neutralize an acid load than on restriction of entry of acid between the cells. Neutralization of acid occurs at the surface due to net HCO₃⁻ secretion and also in the paracellular pathway since the epithelial cells can produce buffer or absorb H⁺. However, once damage has occurred, this paracellular pathway is shorter or more open and the lesion is consequently more sensitive to acid than the normal epithelium.

The measured average net flux of bicarbonate is about 78 μmol/30 min/10 cm in the human oesophagus.³¹⁻³³ It appears to be subject to regulation by muscarinic receptors, responding to the vagal stimulation of acid secretion by the stomach. With reflux of unbuffered gastric contents at a pH of 4.0, there is a load of acid equivalent to 100 nmol/mL. If 10 mL of gastric contents at that pH is refluxed each min for 30 min and spreads over a length of 10 cm, 30 μmol of acid is presented to this length of the oesophagus in that 30 min time span. This is within the average neutralizing ability of the secreted HCO₃⁻. Reflux at pH 4.0 should therefore be largely neutralized at or close to the oesophageal surface. This calculation may explain the results of the above analysis of the ideal pH elevation for the most rapid healing of oesophageal lesions.

At a pH of 3.0, there is 10-fold more acid and 300 μmol of acid fluxes into the lower oesophagus with 10 mL refluxing each minute. This is beyond the capacity of net HCO₃⁻ secretion to neutralize the HCl. Acidification of intercellular spaces is likely, which may result in pain in some individuals. Epithelial cell pH may remain within viable limits given adequate Na⁺/H⁺ or Cl⁻/HCO₃⁻ exchange.

At pH 2.0, the limits of the HCO₃⁻ response are greatly exceeded and the intercellular spaces will acidify even more and to a deeper level. At this stage, the ability of the epithelial cells to maintain constant intracellular pH may be overcome due to the high acid load with resultant erosion. As the more superficial cells are eroded, pepsin may also back diffuse, adding insult to acid injury.

The time necessary for exposure to levels of acid injurious to the leaky epithelium of the oesophagus is not well defined, but pain is clearly related to access of acid to the pain fibres of the lower oesophagus. Hence frequent episodes of heartburn, even in the absence of a visible lesion, are, in the era of proton pump inhibitors, unnecessary for the patient and may predispose to Barrett's oesophagus.

Standard dose proton pump inhibitors, i.e. 20 mg omeprazole, 40 mg pantoprazole, 30 mg lansoprazole and 20 mg rabeprazole, will, in most cases, result in healing of oesophagitis. However, they are less effective in pain relief. Further, given the plasma kinetics of the drugs and the time of onset to steady state, more rapid pain relief is achieved by giving the drugs twice a day, associated with meals. Thereafter, it is generally possible to reduce the dose if maintenance of healing is the sole objective. However, if the sole objective is ablation of symptoms, then the twice-a-day regimen may be continued if symptoms persist on once-a-day therapy. Many studies will have to be carried out to see whether prevention of symptoms decreases the likelihood of oesophageal cancer.

Associated with acid reflux, there may be damage to the larynx, resulting in laryngitis and perhaps also damage to the bronchi, with exacerbation of asthma. Other situations in which it is desirable to prevent reflux are perhaps in intensive care and peri-surgically. Here, larger doses of proton pump inhibitors can be given, since this is generally acute rather than chronic care and, if available, intravenous bolus therapy should prove superior to similar modalities using H_2 -receptor antagonists.

GERD and *H. pylori*

As eradication of *H. pylori* becomes more widespread, data have started to emerge relating the eradication of *H. pylori* to a decrease in the efficacy of proton pump inhibitors.³⁴ The decrease in efficacy of the proton pump inhibitors is irrelevant with eradication in the treatment of duodenal and *H. pylori* induced gastric ulcers, since these are acute diseases with acute treatment. In the case of GERD, however, proton pump inhibitor treatment is chronic and if indeed there is a loss of efficacy of proton pump inhibitors, translating into a loss of control of oesophageal acid exposure, there is the potential for increased symptoms, perhaps recrudescence of erosions and Barrett's oesophagus.

The presence of chronic *H. pylori* infection of the antrum usually results in elevated serum or plasma gastrin levels due to the relative elevation of antral gland pH, presumably due to the NH_3 generated by bacterial urease present within antral glands. Further, when rates of acid secretion are low but intragastric acidity is high, such as at night, there may be sufficient NH_3 generation to partially neutralize gastric contents.

For example, although the average stimulated acid output is about 25 mmol HCl per hour secreted by the H, K ATPase, most of that acidity is neutralized by food and its digestion. At night, the volume of secretion drops drastically but there is also no food buffering.

Let us assume that nocturnal pH is close to 1.0, with a nocturnal fluid volume secreted at 10 mL per hour in the absence of therapy. This implies secretion of 1 mmol HCl per hour into 10 mL to give this a value of pH 1.0. With inhibition of acid secretion at night to elevate intragastric pH from pH 1.0–2.0 by chronic treatment with proton pump inhibitors, acid secretion at night has fallen to 0.1 mmol/h. If the gastric load of *H. pylori* is able to generate ≈ 0.1 mmol NH_3 per hour, then with proton pump inhibitor treatment, net acid production will be significantly lower in infected patients. Hence a pH elevation to perhaps 3.0 will be observed in *H. pylori*-positive patients under proton pump inhibitor therapy. Eradication of *H. pylori* will then result in a lower pH being observed with proton pump inhibitor treatment, namely the usual pH 2.0 between 02:00 hours and 04:00 hours. This may be the simplest explanation for the finding of increased nocturnal gastric acidity after eradication, provided a patient is undergoing proton pump inhibitor therapy. Antral infection predisposes to duodenal ulcer and duodenal ulcer patients often have higher than normal acid output, hence, with antral infection, it is unlikely that *H. pylori* actually reduces acid secretion.^{32, 33} Neutralization by the NH_3 produced seems the more likely explanation. Elimination of this neutralization will lead to a higher intragastric acidity and the potential for a greater acid load to the oesophagus.

For effects to be seen on pH during the day, other factors could also be involved, such as direct inhibition of acid secretion by *H. pylori*, since the usual stimulated acid rate would not notice ammonia production by the organism. However, observations that even intragastric pH during the day is affected by infection is an argument that more than neutralization is happening, since the volume of acid secreted during the day is much greater than that at night and insufficient NH_3 production is anticipated to neutralize this amount of acid.

The observation that proton pump inhibitors are less effective at controlling intragastric acidity after eradication has been used as an argument against eradication for the sake of the health of the oesophagus.^{34, 35} Additionally, the epidemiological observation of a

decreasing rate of infection by *H. pylori* has been used as an explanation for the increased incidence of GERD in the last several years in Europe and the USA. The physician, in deciding to treat *H. pylori* infection, has to decide between a perceived increased risk of Barrett's, compared to the risk of duodenal or gastric ulcer or gastric cancer.

Duodenal ulcer

The vast majority of these ulcers are related to *H. pylori* infection of the antrum, although the mechanism whereby infection weakens the duodenum is not known. In general such patients have higher than normal acid secretion due to a higher than normal parietal cell mass, caused at least in part by the hypergastrinemia induced by the infection.^{36, 37} Prior to recognition of the role of infection, either H₂-RAs or proton pump inhibitors were the treatment of choice, with proton pump inhibitors resulting in a faster healing rate but giving equivalent healing after 8 weeks. In certain groups of patients, such as smokers, proton pump inhibitors have a larger advantage over H₂-RAs. It is now customary to eradicate *H. pylori* in all infected patients with symptomatic duodenal disease or with a history of duodenal ulcer. Proton pump inhibitors in triple therapy are more effective than H₂-RAs in similar regimens. Mostly, duodenal ulcers without infection are iatrogenic, resulting from ingestion of drugs such as NSAIDs. The frequency of this disease is decreasing presumably because of the decrease in the incidence of *H. pylori* infection. Zollinger–Ellison syndrome due to gastrin secretion from gastrinomas also results in duodenal ulcers. High dose proton pump inhibitor therapy is required to suppress acid secretion sufficiently in these patients.

Gastric ulcer

Ulcers in this region are also due either to *H. pylori* infection or to ingestion of NSAIDs. Occasionally, gastric ulcers may co-exist with duodenal ulcers in the Zollinger–Ellison syndrome. For the former, eradication is mandatory whereas in the latter instance, cessation of consumption of NSAIDs which inhibit cyclooxygenase-1 is desirable. If such drugs are taken, proton pump inhibitors are generally effective at prevention of the disease although the dose of proton pump inhibitors should be tailored to the frequency and level of NSAID

usage. Thus far, cyclooxygenase-2 inhibitors cause less gastric pathology and may supersede cyclooxygenase-1 inhibition as pain medication. Zollinger–Ellison syndrome is treated with high doses of proton pump inhibitors to reduce acid secretory capacity by > 90%.³⁸ If the source of gastrin can be defined, surgical removal is indicated.

Proton pump inhibitors as diagnostics

Often patients present with a compendium of symptoms that require careful analysis to determine the underlying disease. For example, chest pain arises from a variety of causes and initial acid inhibitory treatment, if effective, can separate acid related disease from other symptoms in the upper abdomen and chest. A large number of patients also present with symptoms in this area, which cannot be directly diagnosed with modern methods including endoscopy. These clusters of symptoms usually end up being classified as due to dyspepsia (non-ulcer dyspepsia) and maybe a third of these patients do have acid related disease, often helped with adequate suppression of acid secretion.

Non-ulcer dyspepsia

This is presentation of upper gastrointestinal problems not related to visible lesions in the gastrointestinal tract, apparently not related to *H. pylori* infection, nor often to acid secretion. Treatment of these symptoms is challenging and no specific therapy involving proton pump inhibitors is generally recommended.

GASTRIC BIOLOGY OF *H. PYLORI*

It was with amazement and disbelief that the world of gastroenterologists and gastric physiologists heard of an article in Lancet in 1983, claiming that at least the recurrence of duodenal ulcer was due to gastric habitation by an organism called at the time *Campylobacter pylori*.⁸ Perhaps on reflection, some conceded that with the use of H₂-receptor antagonists and the recognition that maintenance was necessary to prevent recurrence, there was an underlying cause that could be due to a chronic bacterial infection, even though it was hard to accept that an organism could occupy a stomach with normal or even supra-normal acid secretion. It required several clinical studies to convince an audience of largely clinical researchers that, indeed,

a bacterium contributed not only to the recurrence but also to the occurrence of peptic ulcer disease. The advent of sequence analysis allowed a more accurate classification to the *Helicobacter* rather than the *Campylobacter* species. Further study even showed an association with a fourfold increase in gastric cancer.³⁹ Within a decade of its discovery, plans were being laid to eradicate the organism, first with bismuth, then with dual therapy involving omeprazole and amoxycillin, then with triple therapy involving proton pump inhibitors and metronidazole or amoxycillin along with clarithromycin. This was followed by even quadruple therapy; ranitidine subcitrate, amoxycillin, clarithromycin and bismuth. The most frequently used regimen today is triple therapy, proton pump inhibitors and two antibiotics taken twice a day for seven, 10 or 14 days. With such a regimen, eradication reaches from the 80th to the 90th percentile. However, this regimen is applied largely to symptomatic patients and not always to patients who test positive for *H. pylori* but are otherwise asymptomatic. Although genes expressed by the pathogenicity island correlate with outcome of infection for the most part, there is still a lack of understanding of why 80% of infected individuals never suffer distress from the infection.⁴⁰

From a commercial point of view, since peptic ulcer disease is disappearing and eradication is an acute indication, the market for a new drug dedicated to eradication is limited. Some take the view that if eradication were simplified and *H. pylori* targeted, all infected individuals would be treated and this of course makes for a large market for some years to come. Here, we are going to explore at least one of the unique features of this organism, to investigate potential unique targets for eradication therapy and rationalize the combination of antisecretory therapy with antibiotics, the current standard for eradication.

The organism

H. pylori is a Gram-negative motile organism unique in its ability to colonize the normal human stomach. It is characteristically spiral, with six flagella at one pole. Its genome has been sequenced and it has about 1500 open reading frames encoding for a variety of proteins, about two-thirds of known function by homology with other proteins.

Bacteria have adapted remarkably to variations of pH in their environment. Bacteria adapted to thriving at

close to neutral pH environments are neutralophiles; those adapted to thriving in acid are acidophiles. Gram-negative organisms, such as *H. pylori* have an outer membrane penetrated by transport proteins such as porins which allow several classes of molecules to pass across this membrane, a cell wall made up of proteoglycans contained within the periplasm and the inner membrane which functions in much the same way as the plasma membrane of mammalian cells. A major function of this inner membrane also resembles that of the inner membrane of mitochondria and chloroplasts. Embedded in this membrane are various oxido-reductases or redox systems which generate a membrane potential, inside negative, and a proton gradient, acid outside. The sum of the potential and the proton gradient is the energy gradient (proton motive force) across the inner membrane, used by the bacteria to synthesize ATP and to actively transport ions and solutes. In general, this gradient is maintained relatively constant so that when the pH gradient inwardly increases, the negative interior membrane potential decreases and vice versa.

Another important property of the inner membrane is the use of the membrane potential to aid in proper protein folding, according to the inside positive rule. Exactly how this works, in terms of charge distribution of charged amino acids that are inserting into the membrane, is not known, but in the absence of an adequate membrane potential, even with an adequate proton motive force, bacterial protein synthesis stops.

Acidophiles therefore always have a large inward acid or pH gradient and therefore have either a low or interior positive membrane potential. Neutralophiles are able to adapt their membrane potential to allow survival in a range between a medium pH of 4.0 to 8.0, where the potential varies from close to zero at pH 4.0 to ≈ 200 mV at pH 8.0. Neutralophiles die when the environmental pH falls below 4.0, since the porins protecting the periplasm from acid are highly permeable to protons and a periplasmic pH < 4.0 is rapidly lethal to these organisms because the inner membrane is unable to exclude protons at a concentration of 10^{-3} M.⁴¹

Many of these organisms, such as *Yersinia enterocolitica* or *salmonella*, are able to survive gastric transit. Only one organism, *H. pylori*, has adapted itself to allow not only survival in, but also habitation of, the human stomach. Therefore, the organism as an acidophile or as a neutralophile, has adapted to regulate its periplasmic pH appropriately in the gastric environment. As a function of its regulation of the pH of its periplasm it

must also have adapted its inner membrane proteins to function best at whatever pH is present in the periplasm.

H. pylori grows best between a medium pH of 6.0 and 8.0, hence is not an obligate acidophile.⁴² Median intragastric pH over a 24-h period is 1.4. Data generated by measurement of gastric surface pH using microelectrodes suggest that there is a standing pH gradient resulting in the surface approaching neutrality when medium pH > 2.0. The gradient disappears when the pH falls to less than 2.0.⁴³ More recent measurements using dyes and confocal microscopy have disputed the presence of a pH gradient at the gastric surface even at higher pH values.⁴⁴ Whichever set of pH measurements is correct, it is certain that the organism has to survive an acid environment lethal to ordinary neutralophiles. For colonization it also has to grow at that pH.

Measurement of inner membrane potential and cytoplasmic pH in *H. pylori* as a function of medium pH (using fluorescent dyes) has shown that its behaviour was that of a classical neutralophile, with a proton motive force (pmf) of ≈ -200 mV, maintained between pH 4.0 and 8.2, and with an irreversible loss of proton motive force at a pH < 4.0 and > 8.2.^{41, 42} The loss of proton motive force at pH < 4.0 occurs within a few seconds; the loss at higher pH is more gradual. Measurement of protein synthesis by the organism showed that there was virtually no protein synthesis at a pH < 6.0 and therefore no growth.⁴² The organism must have developed mechanisms to survive gastric acidity that also allow growth in the stomach.

H. pylori is motile with about six flagella at one end of the organism. Flagellar motion appears to be essential for infection of animal models, since deletion mutants of the flagellar system are also unable to colonize.⁴⁵ The other components for acid resistance that have been identified by knock out strategies are genes within the urease gene cluster.

The urease of H. pylori

Urease hydrolyses urea to CO₂ and 2NH₃. With carbonic anhydrase present in the genome of *H. pylori*, this CO₂ generated will be able to increase the buffering capacity of the cytoplasm to help resist intrabacterial alkalization due to the generation of NH₃. The NH₃ produced can act as a buffer after leaving the bacterial cytoplasm and entering the bacterial periplasm, by the formation of NH₄⁺. Alternatively, it can extend its buffering or acid neutralizing activity by exiting the

outer membrane and reacting with H⁺ in the medium. This enzyme, which is synthesized constitutively by the organism accounting for about 15% of protein synthesis, is an obvious candidate for the acid resistance mechanism of *H. pylori*.

The urease of *H. pylori* is a two-sub-unit enzyme, Ni²⁺ containing, and inhibited at high affinity by phosphoramidates such as fluoroamide. It is found mainly in the bacterial cytoplasm (intrabacterial compartment) and adhering to the outer surface of the organism. It has a neutral pH optimum and is inactive at pH < 4.5. This defines a problem for hypotheses that invoke the surface urease as being responsible for acid resistance since it is unlikely that the organism is found in the stomach at pH levels always greater than 4.5. However, if intracytoplasmic urease were always active, then at times when intragastric pH were neutral, such as in the digestive phase, cytoplasmic alkalization would be lethal for the organism. This dilemma was resolved by measurement of urease activity in intact organisms, compared to surface or soluble urease, as shown in Figure 6.^{41, 42}

The total urease activity of the bacteria is low at neutral pH but undergoes about 10-fold activation between pH 6.2 and 5.5, and remains high even down to pH 2.5. This property must depend on intrabacterial urease which is also shielded from the acidity of the medium by the inner membrane of the organism. The means which *H. pylori* has used to acid activate intrabacterial urease has been identified.

The urease gene cluster

There are seven genes within this cluster. *UreA* and *ureB* are the structural sub-units of the urease, whereas *ureE*, *F*, *G* and *H* are thought to be accessory genes necessary for formation of an active urease by insertion of Ni²⁺ into the *UreA*, *B* protein complex. Their removal results in loss of urease activity. *UreI* is not essential for active urease biosynthesis and is predicted to be a polytopic integral membrane protein from its amino acid sequence. Urease negative mutants have been unable to colonize a variety of animal models and deletion of *ureI* also prevents colonization of the mouse stomach (Figure 7).⁴⁶

Urease activation and acid resistance

An explanation for the acid activation of intrabacterial urease could be that urea access to the intrabacterial

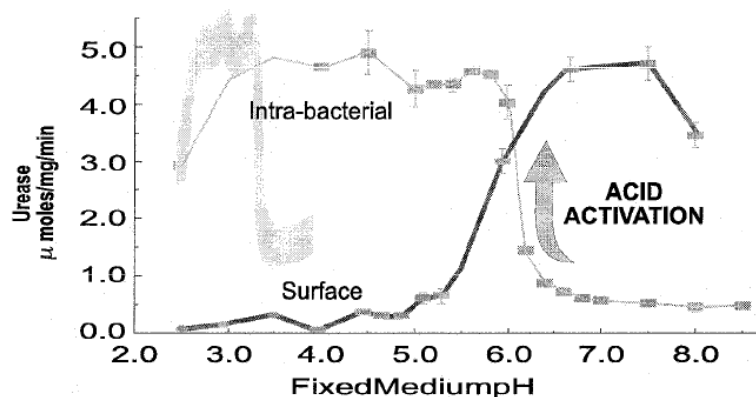


Figure 6. The pH profile of surface and intrabacterial urease under strong buffering conditions preventing any change of pH during urease measurement. The right hand curve shows the pH-activity curve of surface or free urease and the left hand figure the pH-activity curve of intrabacterial urease. Evidently, intrabacterial urease increases its activity as pH falls, showing the presence of an activation process.

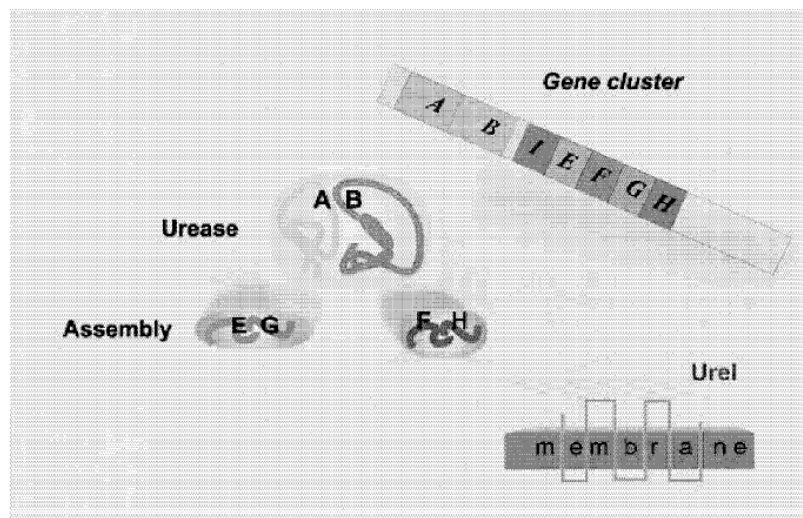


Figure 7. The urease gene cluster showing that *ureA* and *ureB* encode the structural sub-units of urease, and that *ureE-H* encode accessory genes for active urease biosynthesis. UreE and G and UreF and H proteins form pairs and in turn associate with ureB to form the complex that synthesizes active urease. Urel is predicted to be a membrane inserted multimembrane segment protein shown to catalyse urea entry under acid conditions.

urease is rate limiting. The usual concentration of urea in gastric juice is ≈ 1 mM. With a urea permeability across an unmodified bilayer of $\approx 4 \cdot 10^{-6}$ cm/s the rate of permeation of this concentration of urea would not suffice for acid resistance at normal intragastric pH. Therefore, is Urel responsible for acid

activation of the intrabacterial urease and if so, how is this achieved?

In microbiology, the first approach is knock-out of the gene followed by investigation of the properties of the mutant. In non-polar *ureI* knockouts, acid activation of intrabacterial urease disappears, although total urease

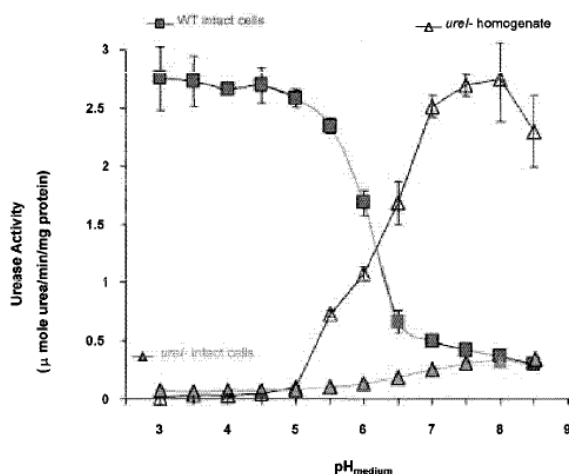


Figure 8. Urease activity pH profiled in wild-type and *ureI*-mutants. In red is the typical wild-type activity curve and in black the normal urease activity of the homogenized mutants. The normal level of urease shows that the mutation is non-polar. In cyan is the pH-activity curve of the mutated intact organisms showing loss of acid activation of intrabacterial urease.

activity is unaffected. Hence UreI is responsible for the acid activation of this compartment of bacterial urease (Figure 8).

If UreI is an acid activated urea transporter, its transport properties can be most readily assessed in *Xenopus* oocytes, where it is expressed following injection of the mRNA for this particular gene. Oocytes express neither urease nor urea transport. Experiments showed that the eggs expressing *ureI* had uptake of urea only at pH 5.0 and not at pH 7.0. Even more remarkable was the pH profile of urea transport in these eggs as shown in Figure 9. There was virtual identity with the pH activation curve of intrabacterial urease.⁴⁷ Other properties of UreI in oocytes suggested that it was an H⁺ gated urea channel, allowing very fast access of gastric juice urea to intrabacterial urease, even at the 1 mM concentration found in gastric juice. It was determined to be a six transmembrane segment inner membrane protein, with the second of the two large periplasmic loops acting as a H⁺ switched urea gate. Functionally it is perhaps at least a dimer.

Hence UreI increases membrane permeability to urea allowing access of urea to the major location of urease. Absence of activity at neutral pH prevents expression of high urease activity at neutral pH, blunting alkalinizing

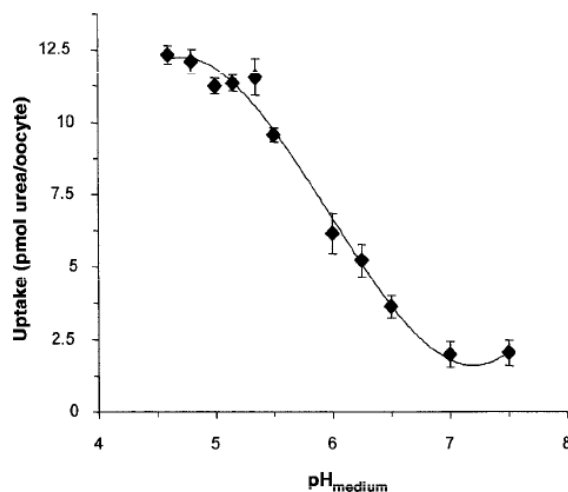


Figure 9. The pH profile of ¹⁴C-urea uptake into *ureI* expressing oocytes. Half maximal activation was at \approx pH 6.0, as found for intrabacterial urease.

toxicity at neutral pH. If this is truly the action of UreI protein, the effect of *ureI* deletion should be overcome at urea concentrations high enough to permeate the membrane at a rate adequate for generation of sufficient quantities of NH₃ for acid resistance. When wild-type *H. pylori* are exposed to a medium at pH 2.5, there is no survival unless urea is present; 0.7 mM urea suffices for 50% survival with 30 min exposure to pH 2.5. The *ureI* knock out mutants require 200 mM urea for 50% survival at this pH (Figure 10). Thus UreI increases inner membrane permeability to urea \approx 300-fold. In the presence of 1 mM urea, there is rapid elevation of the pH of the periplasm at acidic pH as shown in confocal microscopy of the organisms in the presence of BCECF free acid.

At the time of writing this review, it appears that the urease/ureI system on its own can account for acid resistance of the organism and is necessary for colonization. This may not be the only factor required for bacterial colonization. As discussed earlier, absence of motility also prevents colonization and adhesion to gastric cells may also be a requirement. However, it would seem that if acid resistance were eliminated by a selective inhibitor of UreI, thus far a unique protein only expressed in *Helicobacter* spp. that colonize the stomach, a novel therapeutic agent for eradication would be available.⁴⁷ This would target the acid resistance mechanism of *H. pylori*, as illustrated in Figure 11.

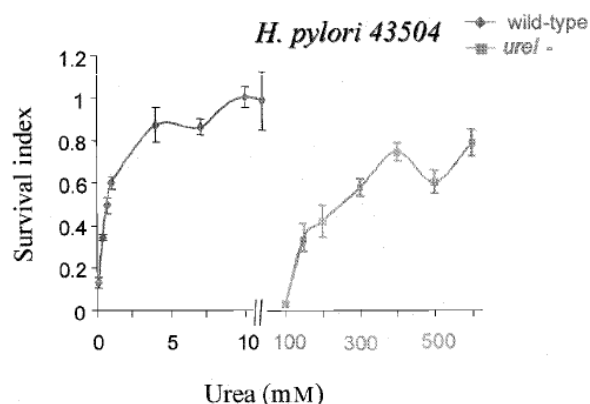


Figure 10. Acid resistance as monitored by survival of exposure to pH 2.5 for 30 min of wild-type and *ureI*-organisms showing that 0.7 mM urea is required for 50% survival and that 300 mM urea is required for this survival in the *ureI* knock-out strain. This is consistent with *ureI* acting as a urea transporter under acidic conditions.

Urease and neutral pH intolerance

The absence of *H. pylori* in patients with pernicious anaemia, or in other neutral regions of the mouth or the gut, requires consideration. The organism is lytic and

urease becomes bound to its surface. At this location, the urease is inactive in acid but active at neutral pH. Additionally, there is slow diffusion of urea into the organism. Elevation of the environment to a pH > 8.2 is lethal to the organism, and with 1 mM urea at neutral pH, this degree of elevation of pH can be achieved. Hence the neutral pH optimum urease produced by the organism and its intrabacterial and surface location allows acid resistance but also enables neutral pH intolerance. This would reduce exposure of the organisms antigens to the potent antibody-producing system of the intestine and perhaps improve its chances of infection.

Current therapeutic approaches

The pathogenicity of *H. pylori* is well recognized, if not well understood. There is a unique genetic locus, *cag*, which contains 31 genes associated with pathogenicity and hence *cag* is a pathogenicity island. Some of the genes in this region are thought to enable type IV secretion, i.e. direct transfer of bacterial protein into cells to which the bacteria adhere.⁴⁸ Expression of various proteins is also often associated with virulence, such as CagA, VacA and Ice.^{48–51} The function of these

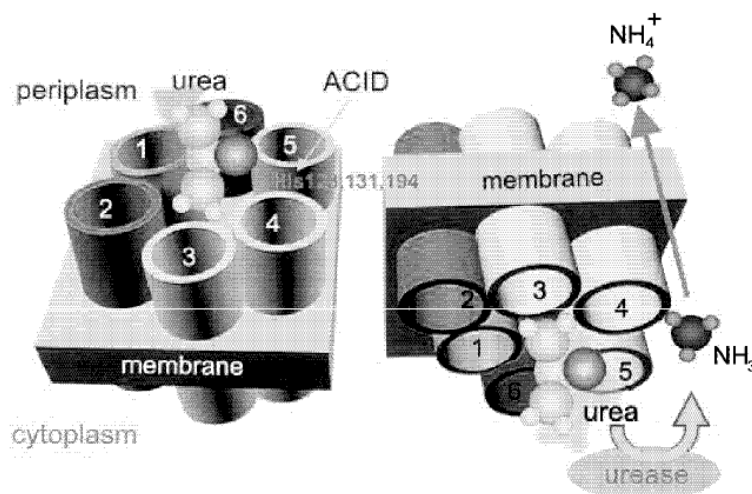


Figure 11. A key mechanism for acid resistance of *H. pylori* in a three dimensional model assuming function as a monomer. Both H^+ and urea enter the periplasmic space via outer membrane proteins, porins, that are permeable to a large variety of small and large solutes. When the pH falls below about 6.0, *ureI*, a urea transporter, is activated by protonation of a histidine (His 123) in the second periplasmic loop. Urea enters the bacterial cell very rapidly via active *UreI*. Intra-bacterial urease hydrolyses the urea producing NH_3 , which diffuses rapidly across the inner membrane and neutralizes the acidity of the periplasm forming NH_4^+ . Between pH 2.5 and 6.0, 1 mM urea suffices to set the pH of the periplasm to 6.2, enabling not only survival but also growth in the stomach if other factors are favourable.

proteins is not known, but a consequence of infection is secretion of IL8 and other cytokines which induce inflammation of the mucosal site at which infection is occurring.⁵² The production of NH₃ may also play a role in the gastritis that inevitably follows infection. Although arguments persist as to the necessity of eradication of *H. pylori* in the asymptomatic patient, many agree that infected patients with upper gastrointestinal symptoms should receive eradication therapy. Such therapy is reasonably complicated, requiring the co-administration of several drugs for 7–10 days.

Thus a proton pump inhibitor is given in conjunction with amoxycillin and clarithromycin on a twice-a-day basis. Antibiotics alone or proton pump inhibitor alone are poor eradicators of the organism. Thus, these two drug classes synergize to remove the organism. Amoxycillin depends on cell wall biosynthesis for its bactericidal action, wherein it binds to critical proteins involved in proteoglycan synthesis. The absence of appropriate synthesis of the cell wall during division results in bacterial death. Clarithromycin inhibits protein synthesis by binding to a site on 23S RNA and results in bacteriostasis when administered. Resistance to clarithromycin is increasing but that to amoxycillin is so far rare. An alternative, metronidazole, is difficult to tolerate and there is considerable resistance to this antibiotic.

Intragastric urea can protect the organism from gastric acid but must also be used to elevate periplasmic pH to 6.2 or above to allow protein synthesis and growth. At the overall pH of gastric contents, perhaps only a minority of the bacteria inhabiting the stomach achieve a pH of ≈ 6.2 and proceed to grow. However, with proton pump inhibitor treatment, where the average pH of the stomach is elevated by 2 or more pH units, more bacteria will be able to grow in the presence of urea. This seems a likely explanation for the synergism between proton pump inhibitors and these antibiotics. Hence co-therapy with proton pump inhibitor + amoxycillin + clarithromycin is the appropriate, current standard for eradication.

Future therapeutic approaches

With the recognition that *H. pylori* is indeed a significant pathogen increasing the risk of serious upper gastrointestinal disease, if there were simple eradication by a single drug, we believe that therapy would become universal. The risk of exacerbation of GERD and acid

reflux, resulting in transformation of the oesophageal epithelium to a pre-neoplastic condition is outweighed by the risk to the stomach and the duodenum. In particular, acid reflux can be controlled by appropriate dosage of proton pump inhibitors or even laparoscopic fundoplication and hence the risk of generation of Barrett's oesophagus can be minimized.

One approach to the discovery of a single eradicator drug is illustrated by the story of ureI, largely based on intuitive reasoning by several laboratories.^{53–55} There may well be other genes necessary perhaps for colonization rather than acid resistance, also unique to *H. pylori*. For example, there are genes apparently required for adhesion to the gastric epithelium, probably necessary for colonization. These can be discovered by intuition or by systematic knock-out of every gene of the organism and determination of its essentiality for colonization.⁵⁶ Perhaps a more rapid approach is to determine genes uniquely expressed *in vivo* and to use these as targets for eradication. It may be relatively straightforward, with modern high throughput screening methods, to discover compounds able to target, be it ureI or other proteins of *H. pylori*. It may be a more difficult challenge to generate gastric targeted drugs based on these novel structures.

Another alternative which is being explored is the use of vaccination, in particular oral vaccination. At first it was thought that administration of bacterial urease in a transportable form with cholera toxin as the transporter might suffice based on data showing not only prevention but reduction of infection in mice.⁵⁷ However, it appears that neither this particular antigen nor the transporter selected will be useful for human eradication.⁵⁸

CONCLUSION

It is interesting to speculate on events in the next century which will guide novel therapeutic approaches to acid related diseases. A major change will be the advent of the complete human genome and analysis using chip array or filter hybridization technology. This will discover genes expressed at different levels in disease-prone, compared to healthy, individuals. If such genes exist in the human antrum or fundus, they may provide a guide to the next generation of therapeutic agents designed to generate a healthy stomach. If there are abnormally expressed genes in the lower oesophageal sphincter, these may help normalize the diseased

entity responsible for GERD. At a simpler level, a similar analysis applied to *H. pylori* infections which result in disease and in asymptomatic commensality may provide a clearer insight as to the pathogenic mechanisms associated with specific genes of the organism inhabiting the stomach. What is certain is that our understanding of disease in this region will undergo a change in the next quarter century, as major as that which has happened in the last quarter century. A concluding quotation from the American writer, H. L. Mencken, may be appropriate for ending this review:

Life is a struggle, not against sin, not against Money Power, not against malicious animal power, but against hydrogen ions.

ACKNOWLEDGEMENTS

This study was supported by US VA SMI funds and NIH grant nos DK40615, 41301 and 17294, NIH DK 52274-01.

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