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PARADIGM SHIFTS IN PERSPECTIVE

Gastric Acid-Dependent Diseases: A Twentieth-Century Revolution

George Sachs · Jai Moo Shin · Keith Munson · David R. Scott

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Introduction

Until recently, peptic ulcer disease (PUD) has been a major scourge to humanity, associated with high incidence of morbidity and mortality, the latter due primarily to foregut perforation or hemorrhage. Advancements in the understanding of the pathophysiology and treatment of PUD have included the discovery of gastric HCl secretion by Prout [1], and the realization that PUD only occurred in the presence of gastric acid [2], leading to the pronouncement "no acid, no ulcer." Apart from a strict bland diet [3], only surgery, ranging from partial or total gastrectomy to vagotomy and to selective or highly selective vagotomy successfully reduced gastric acid secretion [4, 5]. In the last quarter of the twentieth century, three major advances took place, completely altering the treatment of gastric acid-related diseases: (1) the development of histamine$_2$ receptor antagonists (H_2RA) as a result of rational drug design; (2) the development of proton pump inhibitors (PPIs), in part serendipitous but also followed by rational drug design; and (3) recognition that infection by *Helicobacter pylori* (*Hp*) is a major causative factor in peptic ulcer disease and even of gastric cancer [6, 7]. In the last two decades, PUD has been replaced by gastro-esophageal reflux disease (GERD) as the major reason for physician consultation due to foregut-related symptoms.

G. Sachs (✉)
Department of Medicine, David Geffen School of Medicine, The Veterans Administration Greater Los Angeles Healthcare System, University of California at Los Angeles, Bldg 113, Rm 324 11301 Wilshire Blvd., Los Angeles, CA 90073, USA
e-mail: gsachs@ucla.edu

G. Sachs · K. Munson · D. R. Scott
Department of Physiology, David Geffen School of Medicine, The Veterans Administration Greater Los Angeles Healthcare System, University of California at Los Angeles, Los Angeles, CA 90073, USA

J. M. Shin
Jai Scientific, Chatsworth, CA, USA

The Regulation of Acid Secretion

Although gastric acid secretion was discovered in the 1700s, its regulation has only been deduced relatively recently. At the time of the introduction of H_2 RAs, gastrin, discovered by Edkins [8] and isolated by Gregory and Tracy, was thought by many to be the major direct stimulant of acid secretion [9]. Nevertheless, the first-generation H_2RAs, cimetidine or burimamide completely inhibited gastrin-stimulated acid secretion in rats [10], indicating that the major secretory action of gastrin was mediated by histamine. Subsequent characterization of the enterochromaffin-like (ECL) cell revealed that gastrin-stimulated histamine release from this master regulator of acid secretion since gastrin stimulation of acid secretion by isolated rabbit gastric glands was completely inhibited by H_2RAs with a 1 nM affinity [11]. Therefore, the lower affinity (10 nM) gastrin receptor expressed in parietal cells is likely involved with trophic effects such as regulation of differentiation or growth rather than with stimulation of acid secretion. ECL cells are also stimulated by pituitary adenylate cyclase-activating peptide (PACAP) but not by acetylcholine, the vagal mediator of acid secretion, which depends on its binding to muscarinic receptor subtype 3 on parietal cells [12]. Pathways regulating parietal cell acid secretion are shown in Fig. 1. Current knowledge substantiates the central role of histamine as a stimulant of

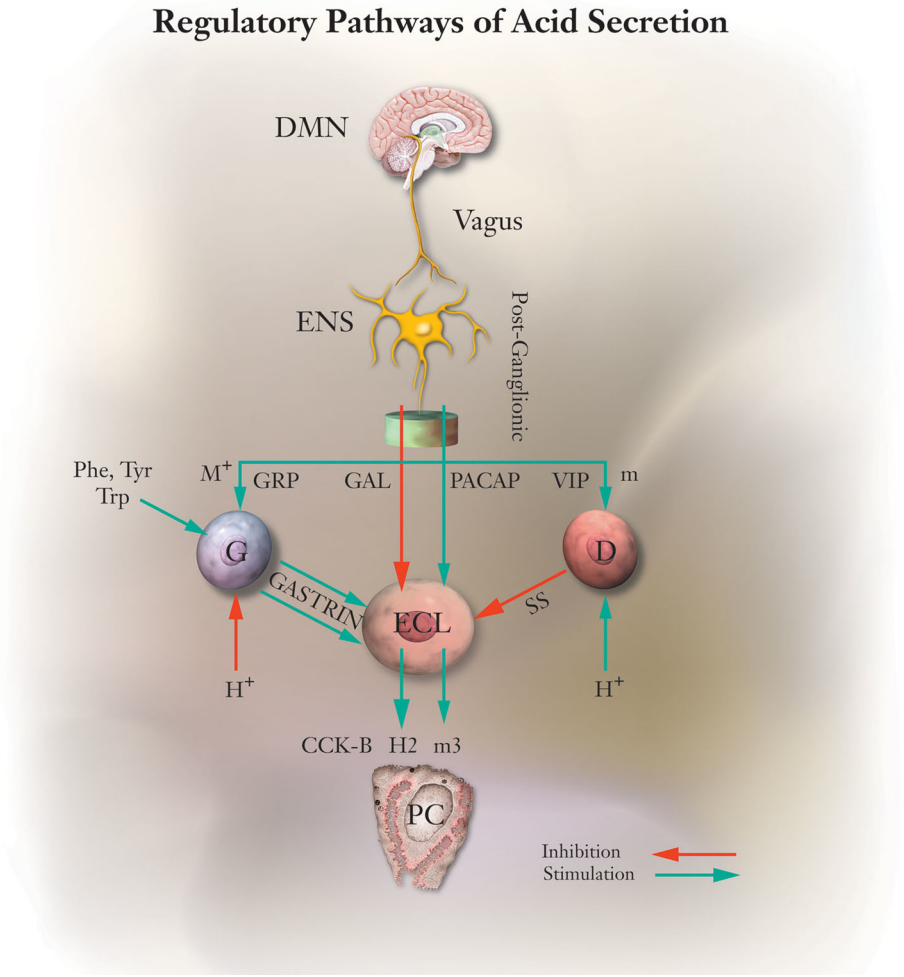

Fig. 1 Regulation of gastric acid secretion showing both activating and inhibitory pathways, neural, endocrine, and paracrine

gastric acid secretion, paving the way for the discovery of H_2RA as a means of down regulating secretion.

Histamine and Histamine Receptor Antagonists

Of the four histamine receptor subtypes, H_1 through H_4, the first of these to be targeted was the H_1 receptor which is responsible for rhinitis and other allergic syndromes. The first successful small ligand receptor antagonist class ever developed was the H_1RA [13] based on the structure of histamine, a known pro-inflammatory ligand and also involved in mucus secretion [14]. Histamine is derived from decarboxylation of histidine, which yields the imidazole ethylamine, known as histamine. Early histamine$_1$ receptor antagonists depended on the modification of the imidazole ring with retention of the ethylamine side chain. Figure 2 depicts the structure of histamine and an early H_1RA pyrilamine. The 3D structure of the H_1 receptor is now available resolved at 3.1 Å resolution docked with doxepin, an early H_1RA that has many unwanted effects such as drowsiness, dry mouth, and arrhythmia. A major

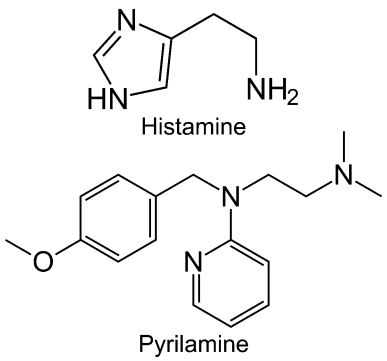

Fig. 2 The structure of histamine and an early H_1 antagonist

site of interaction is the anionic residue asp107 hydrogen bonding with the amine of the ligand (Fig. 3). Second-generation H_1RA add a carboxylate to their structure, facilitating interaction with the lys179 amine, increasing specificity. Other H_1RA also interact with lys191 further improving their selectivity [15], with a marked reduction in adverse effects.

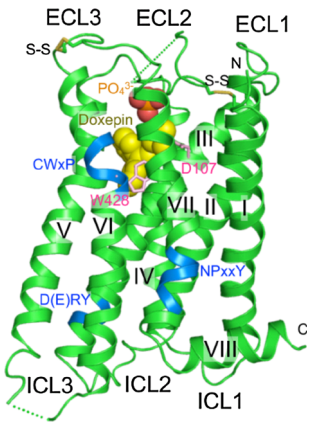

Fig. 3 The 3D structure of the H_1 receptor with doxepin docked, showing interaction with Asp107 H^+ bonding with the amine group of the antagonist

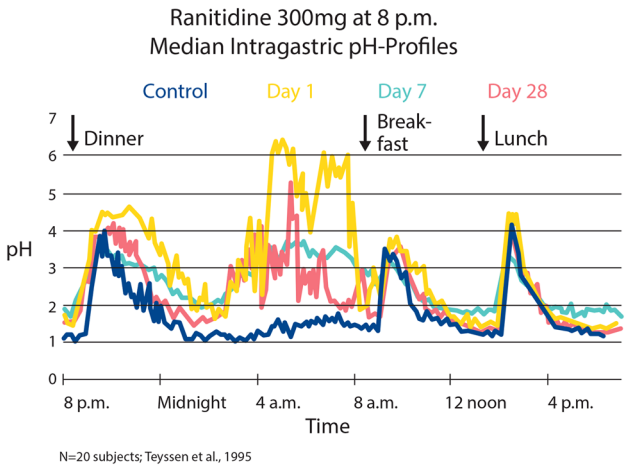

Fig. 5 The effect of once a day, evening administration of ranitidine on intragastric pH showing good night time effect on day 1 with 50 % loss of effect by day 7 through day 28 and little effect during the day requiring BID dosing for more effective control of acid secretion compared to the four times per day recommended for cimetidine. Reprinted with permission from Macmillan Publishers Ltd: Modlin and Sachs [60]

Ash and Schild clearly differentiated the histamine receptors expressed in the circulatory system from those expressed in stomach or uterus [16] concluding that there were at least two classes of histamine receptors. James Black moved from Imperial Chemical Industries (ICI) where he had developed the first β-adrenergic inhibitor, propranolol, to Smith Kline & French (SK&F) in Welwyn (a stately home used during World War II to test miniature submarines since there was a very deep pond on the premises) recruited by Bill Duncan also from Glasgow. Black et al. realized that the secret to developing H_2RAs was not to modify the imidazole ring of histamine as had been done for the H_1RA, but to modify the side chain. I was privileged, as the gastrointestinal consultant for SK&F in Philadelphia, to be sent to Welwyn to review Jimmy Black's progress. Within a seven-year project cycle, 6 years had passed for the H_2 receptor project without success! I was most impressed with the team including Mike Parsons, Robin Ganellin and Graham Durant and submitted a positive report to Peter Ridley and Bryce Douglas the then head of research at SK&F, a Glaswegian like Jimmy. A crucial tool developed by Mike Parsons was the measurement of acid secretion in the rat rather than measuring the degree of ulceration. The next year, since I was aware of the H_2 antagonistic action of burimamide, I was able inhibit histamine-stimulated adenylate cyclase and submit an even more positive report [17], which eventuated in the introduction of cimetidine (Tagamet®) as the first anti-secretory medication indicated for the treatment of peptic ulcer disease [10, 18] (Fig. 4). Cimetidine was rapidly followed by the second-generation H_2RAs ranitidine, famotidine and nizatidine with somewhat different structures and differing duration of action, of which

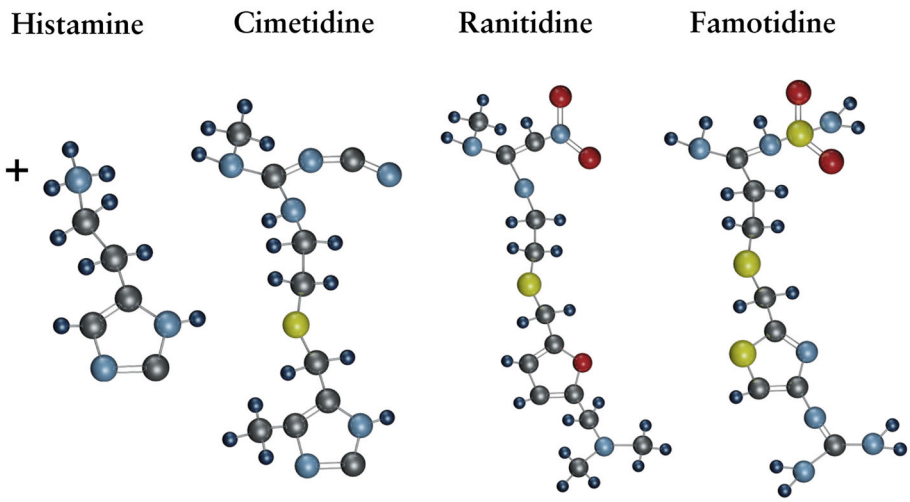

Fig. 4 The structure of cimetidine and two other H_2 antagonists. Reprinted with permission from Macmillan Publishers Ltd: Modlin and Sachs [60]

the most effective is famotidine [19]. The discovery of H_2RAs not only revolutionized therapy of acid-related diseases, but also vastly improved the understanding of the control of acid secretion, as discussed above.

As experience increased with the use of H_2RAs, certain drawbacks to their use became apparent. Firstly, their action was short lived, requiring multiple daily doses. Secondly, all patients exhibited tolerance whereby after one week of treatment, the response was reduced by ~50 % [20] (Fig. 5). Hence, although relatively effective at accelerating the healing rate of duodenal ulcer, they were less effective in the treatment of gastric ulcers. Furthermore, the response in patients suffering from gastro-esophageal reflux disease (GERD) was mostly inadequate [21]. A different means of inhibition of acid secretion was required. Fortunately, by that time, the gastric H^+, K^+ ATPase, the final step of acid secretion that cannot be bypassed, had been discovered. Ganser and Forte showed the presence of a K^+-stimulated ATPase in frog gastric microsomes [22] and Peter Scholes working in Peter Mitchell's laboratory showed that dog microsomes alkalinized the medium in the presence of K^+ in the medium upon addition of MgATP [23].

Reprising our discovery of a K^+-stimulated ATPase in hog gastric vesicles in 1968, we demonstrated details of the H^+ for K^+ ATPase reaction mechanism showing conclusively that it was an electroneutral H^+ for K^+ exchange P_2-type ATPase lacking a K^+ conductance in resting enzyme [24]. This conclusion was based on the lack of effect of lipid permeable ions on either ATP-dependent H^+ or Rb^+ transport and the absence of changes in a membrane potential sensitive dye during H^+ for K^+ exchange in the absence of the K^+ ionophore, valinomycin (24). A later paper used membrane potential dyes such as diethyloxadicarbocyanine or oxonol dyes to confirm electroneutrality [25].

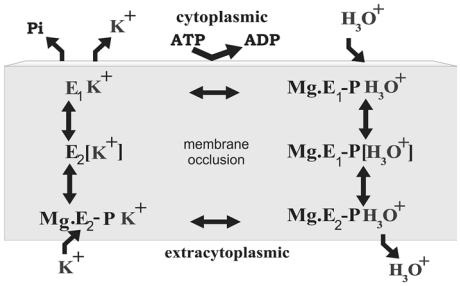

Fig. 6 The catalytic cycle of the gastric H^+, K^+ ATPase. Binding of ATP and hydronium ion results in phosphorylation of the alpha subunit to result in the E_1-P form which then occludes the ion and spontaneously converts to the E_2-P form, releasing hydronium ion to the lumen. K^+ then binds resulting in dephosphorylation and formation of the K^+ occluded form which then releases K^+ to the cytoplasm reforming E_1

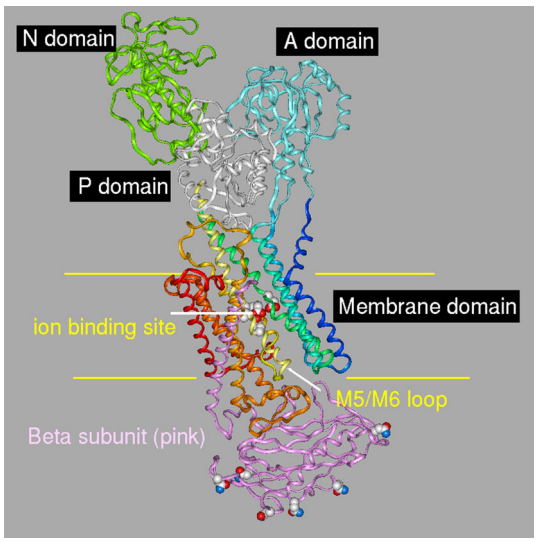

Fig. 7 A homology model of the arrangement of the two subunits of the gastric H, K ATPase in the E2 form where the N and A domains have moved inward to the P domain opening a luminal vestibule allowing K and inhibitor access. The ion binding and glycosylation sites of the beta subunit are also shown

Mechanism of Acid Secretion: The Gastric H^+, K^+ ATPase

HCl secretion by the gastric parietal cell depends on activation of the gastric H^+, K^+ ATPase, termed the proton pump. This enzyme is found uniquely in gastric parietal cells and in renal collecting ducts. It is an electroneutral H^+ for K^+ exchange P_2-type (phosphorylating) ATPase with ten membrane-spanning segments and a β subunit with one transmembrane segment and six or seven glycosylation sites. The catalytic cycle is shown in Fig. 6.

At neutral pH, 2 H^+ are exchanged for 2 K^+ per hydrolysis of 1 ATP, but as the luminal pH falls, the exchange stoichiometry is 1 H^+ per 1 K^+ per 1 ATP. This stoichiometry change is explained by the pK_a of one of the hydronium binding sites which remains protonated at luminal pH < 3.0 [26]. Structurally, it is a heterodimer of an α and β subunit like the Na^+, K^+ ATPase which may exist as a dimeric oligomer, i.e., α2β2 as indicated by stoichiometry of labeling with inhibitors or phosphorylation [27].

Although its 3D structure has not been solved, that of two homologous pumps has been [27, 28]; providing valuable clues about the structure of the H^+, K^+ ATPase by homology modeling, providing in turn a plausible explanation for the mechanism of acid inhibition by PPIs. The cytoplasmic domain has three loops, the N or nucleotide binding domain, the A or activation domain, and P the phosphorylation domain (Fig. 7). The key to transport of H^+ from the cytosol and absorption of K^+ from the lumen are conformational changes induced by phosphorylation of Asp386 by MgATP, which

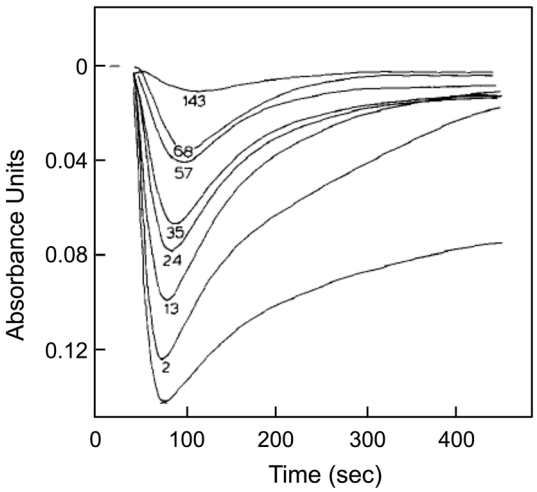

Fig. 8 The effect of ATP addition on acid transport (*down curve*) after different incubation times with a PPI showing delayed inhibition (*up curve*) The progressive effect of time of incubation at pH 7 to activate the PPI (picoprazole)

changes the direction of net ion flux from cytoplasm → lumen to lumen → cytoplasm due to conformational changes in the cytoplasmic domain wherein the N and A domains move close to the P domain with closure of the cytoplasmic access pathway. There is then a spontaneous opening of a luminal vestibule allowing ion access from the luminal side. The E_2 form is illustrated in Fig. 7, showing the placement of the ß subunit and six glycosylation sites. The ß subunit of the sodium pump has only three glycosylation sites which has likely impeded efforts to crystallize the protein.

Activation of the Pump

In the resting parietal cell, most of the ATPase is sequestered in cytoplasmic tubulovesicles, and upon stimulation, it is trafficked to the apical microvilli of the secretory canaliculus [28]. Along with the pump, there is trafficking of the K^+ channel complex KCNQ1 and KCNE2 as well as a Cl^- channel. The channel trafficking is distinct from the trafficking of the ATPase [29, 30].

Proton Pump Inhibitors (PPIs)

This novel acid therapeutic class was initially discovered serendipitously by the Swedish company, AB Hässle during a secondary screen of antiviral compound candidates for anti-ulcer activity in a rat ulcer model. A compound, pyridine 2-acetamide, inhibited gastric acid secretion, even after conversion to the more stable pyridine 2-thioacetamide. Since the imidazole ring was considered essential for the anti-secretory activity of H_2RA, Hässle added an imidazole ring to pyridine 2-thioacetamide based on advice from their gastrointestinal consultant Lars Olbe. Since imidazole was not available, benzimidazole was added to produce pyridine 2-methylthiobenzimidazole. With an added sulfoxide to increase stability, timoprazole, the precursor of all PPIs, resulted. Since timoprazole, unlike H_2RAs, inhibited acid secretion independently of stimulus, it was considered a new class of antisecretory compound. My laboratory presented data showing that a polyclonal antibody against the gastric H^+, K^+ ATPase reacted with extracts of the stomach, thyroid, and thymus. We therefore hypothesized that the compound inhibited the gastric H^+, K^+ ATPase, the final step of acid secretion. Timoprazole did not inhibit purified H^+, K^+ ATPase when net acid secretion was "short-circuited" by the H^+ for K^+ exchange ionophore, nigericin. Since timoprazole was acid unstable, we tested timoprazole against the H^+, K^+ ATPase using conditions that promoted H^+ for K^+ exchange though the ATPase, where the compound inhibited ATP-stimulated

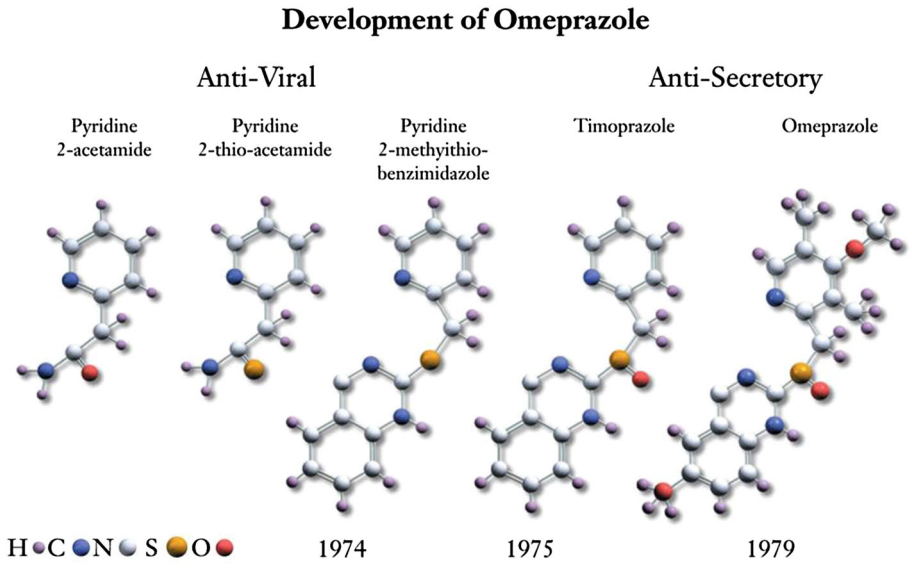

Fig. 9 The pathway to the generation of omeprazole based on inhibition of gastric acid secretion and stabilization to medium pH. Reprinted with permission from Macmillan Publishers Ltd: Modlin and Sachs [60]

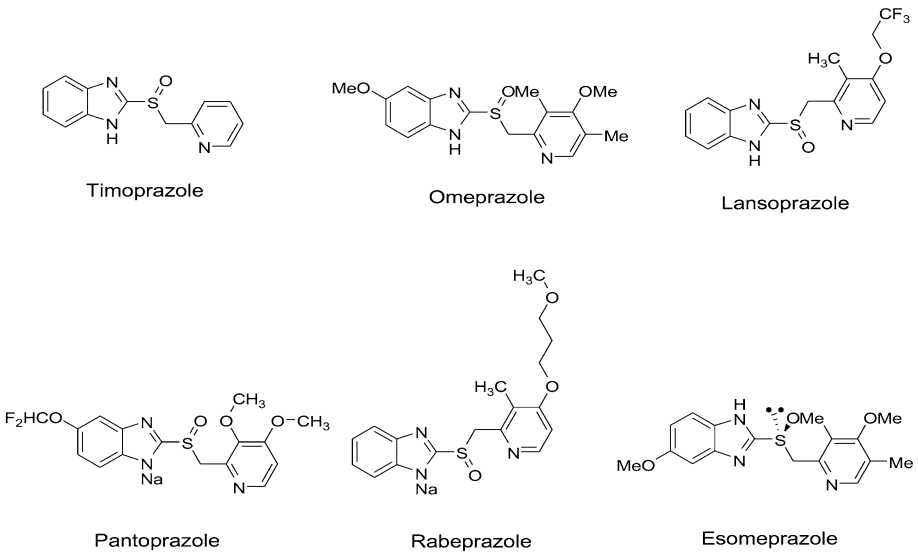

Fig. 10 The currently marketed PPIs

H^+ secretion (Fig. 8) [31]. This and experiments like this indicated that timoprazole was an acid-activated prodrug, serving as the basis for omeprazole, as shown in Fig. 9, with substitution on both the pyridine and benzimidazole rings.

Since they are acid activated, PPIs are usually enteric coated in order to impair gastric drug release. Since there is significant activation of all PPIs at neutral pH, IV formulation of the PPIs pantoprazole and esomeprazole requires that the compound must be dissolved in buffer with pH $>$ 9.0 in order to delay activation immediately prior to administration [32].

During the development of omeprazole, serious concerns were raised by the management of Hässle as to its value, in particular since ranitidine then dominated the antisecretory market. With my [GS] clinical background, my knowledge of its molecular mechanism and potent antisecretory properties, I was convinced that omeprazole would become the market leader in its class, with worldwide sales exceeding US $1 billion annually, which grossly underestimated its peak sales revenue of US $6 billion, with the overall revenue of all drugs in its class exceeding US $20 billion annually. Every 3 months, I attended a meeting of the "PPI team" in a basement room at Hässle, which helped me maintain close collaborative ties between my laboratory and that of Hässle. The team of about 12 scientists was given total freedom by Anders Vedin, the research head at Hässle, to understand every key aspect of omeprazole and to bring it to market with no interference from oversight committees. Many of the team members spent time in my laboratory including Herbert Helander, Thomas Berglindh, Björn Wallmark, Pia Lorentzon, and Karen Gedda. Also, at the time, Byk-Gulden in Konstanz, Germany, allied with SK&F, started PPI development, culminating in the development of pantoprazole (Protonix®) aided by the translational studies of David Keeling and Alex Simon on loan to my laboratory. Also very important at the time was the Astra Hässle marketing manager, Ian Talmage, who was a remarkably talented in the art of product branding. All of these talented individuals have remained lifelong friends.

As phase III trials were underway, rats, but not mice or dogs, developed ECL cell carcinoid tumors, halting the clinical PPI trials, which the involved scientists were convinced was an effect of hypergastrinemia and not primarily drug dependent, supported by data generated by a Hässle team led by Enar Carlsson, which was sufficient to re-start the clinical trials. A key experiment used to support the hypergastrinemia hypothesis was that rats treated with high-dose H_2RA also developed ECL tumors [33]. In contrast to rats, the ECL cell is terminally differentiated in humans, explaining why ECL tumors have not been described in humans receiving long-term, high-dose PPI therapy. Nevertheless, experimental findings prompted the competition to claim carcinogenicity for omeprazole [34].

Since the clinical introduction of omeprazole, several other drugs have entered the market which are also acid-activated prodrugs, sharing similar advantages and disadvantages with omeprazole (Fig. 10). Esomeprazole (Nexium®) is the S-enantiomer of omeprazole which at 40 mg per day compared to 20 mg per day of omeprazole shows a slight benefit in acid control. The most recent entry to the market is the D-enantiomer of lansoprazole (Dexilant®) which is formulated be released immediately and after a ~4-h delay. All of the PPIs in clinical use are activated prodrugs forming a thiophilic reactive group that binds covalently to one or more cysteines on the gastric H^+, K^+ ATPase irreversibly inhibiting the enzyme.

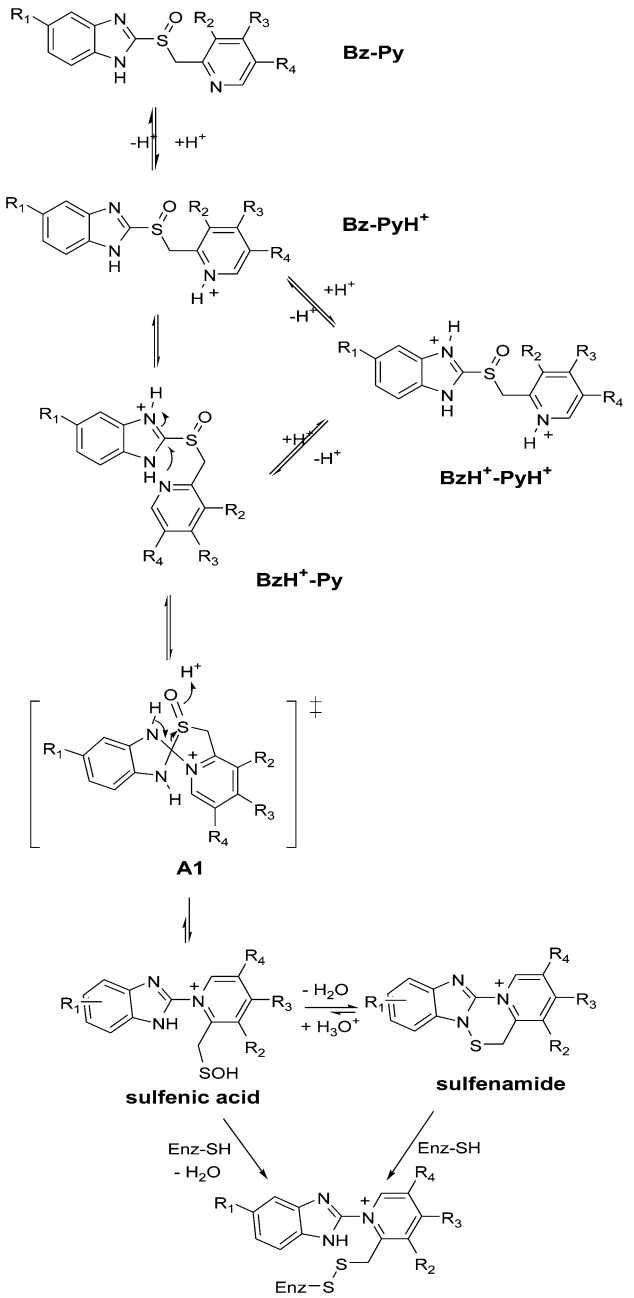

Fig. 11 The pathway of pH activation of a benzimidazole-based PPI. Protonation of the pyridine (pk_a 4.0) allows selective accumulation of the drug in the active parietal cell and then at highly acidic pH of the secreting cell, protonation of the benzimidazole ($pk_a \sim 1$–2) results in rearrangement, forming the thio-active drug

Fig. 12 The binding sites cys813 and cys822 of pantoprazole on the luminal face of the alpha subunit

The mechanism of activation of the PPIs is a remarkable series of chemical steps as shown in Fig. 11, elucidated by Arne Brandström and Per Lindberg at Hässle and refined by Jai Moo Shin in my laboratory [35, 36]. There is still disagreement as to whether the active compound in vivo is the sulfenic acid or the sulfenamide which was the form isolated at Hässle. Please see [37] for a comprehensive review of this area. The mechanism is shown as a general structural form (Fig. 11). The top of Fig. 11 shows the protonation of the pyridine ring with a pK_a between 4.0 (omeprazole, lansoprazole, pantoprazole) and 5.0 (rabeprazole), accumulating the protonated form only in the actively secreting parietal cell, since this is the only space with a pH < 4.0. Below that is shown the protonation of the benzimidazole ring with a pK_a of <2.0 and in brackets is shown the mechanism of activation where the C2 of the protonated benzimidazole ring reacts with the unprotonated fraction of the pyridine moiety rearranging to a permanent thioreactive, cationic, tetracyclic sulfenamide that binds covalently to one or more luminally accessible cysteines of the α subunit of the gastric ATPase. In aqueous solution, the sulfenic acid dehydrates to form the sulfenamide. The cationic sulfenic acid or sulfenamide remains trapped in the parietal cell canaliculus. In the particular case of pantoprazole, cysteine 813 and cysteine 822 become covalently linked (Fig. 12). With other PPIs different cysteines are linked but cysteine 813 is derivatized by all PPIs and must be considered as the central target for this class of drug. It is easy to visualize that binding of a PPI covalently in this region will fix the pump in the E_2 form and inhibits cycling back to the E_1 form.

Thus, since PPIs require ongoing acid secretion in order to be activated, they are administered 30–60 min before breakfast so that peak blood concentrations coincide with maximal H^+, K^+ ATPase activity. In spite of the need for

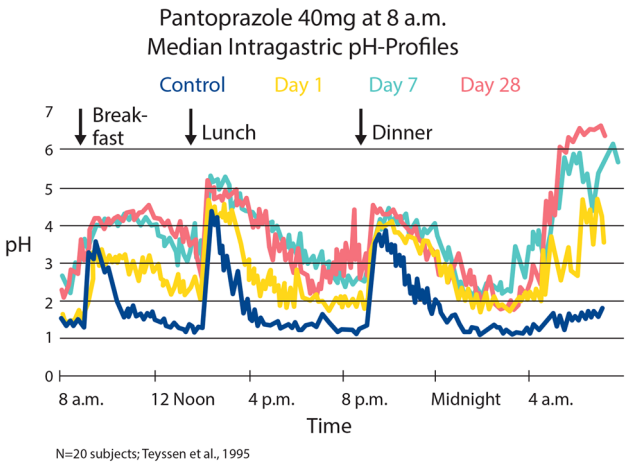

Fig. 13 Intragastric pH profile with PPIs before breakfast. *Arrow* show pH at night <2.0. Reprinted with permission from Macmillan Publishers Ltd: Harder et al. [61]

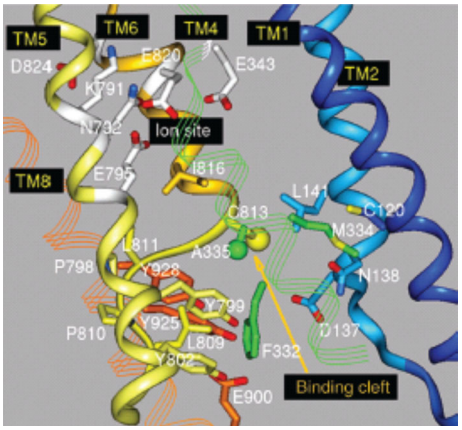

Fig. 14 Higher magnification of the luminal vestibule of the E_2 form of the alpha subunit showing access to cys813 the common covalent binding site of all PPIs

careful timing with meals, pH suppression by PPIs is superior to that of H_2RAs due to lack of tolerance. A typical intragastric pH profile after PPI treatment is shown in Fig. 13, with progressive improvement in acid control with daily dosing. Although significant inhibition of acid secretion is present on the first day of dosing, inhibition improves significantly up to the third or fifth day of dosing, since with covalent labeling of pumps cycling between activity and inactivity, the percentage of inhibited pumps progressively increases. With a half-life of about 50 h, ~25 % of pumps are synthesized *de novo* synthesis/day, hence maximal pump inhibition on once daily dosing can only reach 75 % of maximal acid secretion. As can be seen in the pH profile after multiple treatments of once-daily PPI, intragastric pH still falls to <2.0 in the middle of the night, a finding scarcely affected even with twice daily mealtime dosing, due to the short plasma half-life (~90 min) of all currently marketed PPIs with no drug present when newly synthesized or previously silent pumps become active.

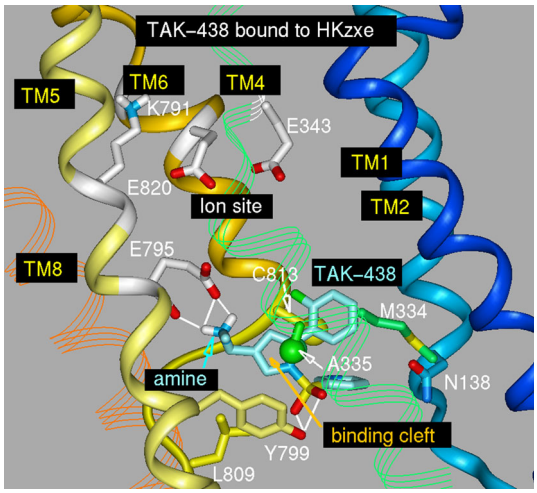

Fig. 15 The modeled binding site of the K^+ competitive inhibitor, TAK-438, binding in the same region as omeprazole

Marketing of Omeprazole

Merck launched omeprazole in the United States in 1989 under the name Losec® which was changed to Prilosec® in the United States a year later in order to avoid confusion with similarly named products. The superiority of omeprazole over H_2RAs was most evident in the treatment of GERD which became, and continues to be, the major clinical indication for PPIs. As a result of a systematic meta-analysis of the correlation of intragastric pH and clinical outcome, maintenance of intragastric pH > 3.0 for 12 h/day optimized the healing of duodenal ulcer, whereas maintenance of pH > 4.0 for 18 h/day optimized the treatment of GERD [38, 39]. At the time of launch, ranitidine had overtaken cimetidine as the major drug used for acid-related diseases largely due to its twice daily dosing compared with four times daily for cimetidine. "Scare" strategies, such as stating that omeprazole permanently inhibited acid secretion, were employed to discredit omeprazole, even though the ongoing synthesis of new pumps and clinical experience did not support this. The climax of the fight came when a letter appeared in the journal *Lancet* claiming that omeprazole induced unscheduled DNA synthesis (UDS) in isolated parietal cells implying that omeprazole was carcinogenic [34]. The technique used, which was developed in my

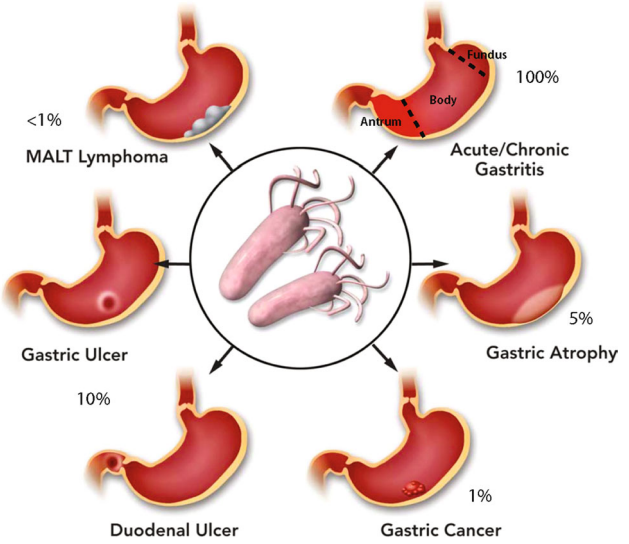

Fig. 16 The outcomes of infection by *Helicobacter pylori*. Reprinted with permission from Macmillan Publishers Ltd: Sachs and Scott [62]

laboratory [40, 41], did not discriminate between surface cells and oxyntic cells. Surface cells are derived from constantly dividing stem cells which continuously physiologically incorporate the DNA precursor thymidine, which is not indicative of UDS. Moreover, the *Lancet* article's author published half of the dose–response curve, whereas the complete curve was bell-shaped and clearly could not represent UDS. Several subsequently published studies did not support the UDS hypothesis, removing any lingering doubts regarding the superiority of PPIs over H_2RAs for treatment of GERD [42].

Current Situation of Clinical Use of PPIs

The PPI class of drugs, though still the most widely used in the acid-related disease market, has been attributed with certain drawbacks over time. For example, 20 % of GERD patients continue to have reflux symptoms despite maximal PPI therapy, due to the presence of poorly suppressed nocturnal acid secretion. A novel sulfonamide prodrug of omeprazole AGN904, with delayed absorption is capable of maintaining intragastric pH > 5.0 for 24 h/day, promises to be effective for the recalcitrant 20 % of GERD patients with prominent nocturnal secretion [43], and also for the eradication of *Hp* which become persistent and

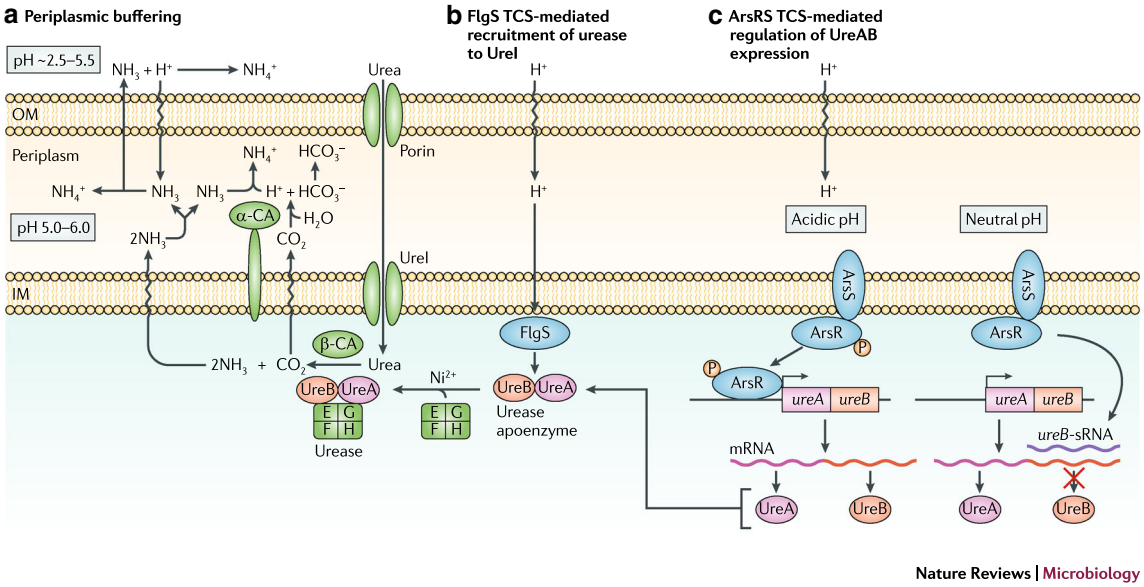

Fig. 17 Periplasmic buffering by *Helicobacter pylori* and its regulation. *a* Urea crosses the outer membrane (OM) and then the inner membrane (IM) through the pH-gated urea channel, UreI, at an external pH < 6.0. Cytoplasmic urease forms $2NH_3 + H_2CO_3$ and the latter is converted to CO_2 by cytoplasmic β-carbonic anhydrase. These gases cross the IM, and the CO_2 is converted to HCO_3- by the membrane bound α-carbonic anhydrase thus maintaining periplasmic pH at ~6.1, the effective pK_a of the CO_2/HCO_3^- couple. Exiting NH_3 neutralizes the H^+ produced by carbonic anhydrase as well as entering H^+ and can also exit the OM to neutralize the medium and thus allows elevation of periplasmic pH to higher than medium. *b* The role of the pH responsive two component system, TCS, FlgS encoded by HP0244. Activation results in recruitment of urease proteins to UreI and the immediate access of urea and outward transport of CO_2, NH_3 and NH_4^+ through UreI increases the rate of periplasmic buffering. *c* A model representing the role of regulation by the TCS ArsRS (encoded by HP0165/HP0166). At neutral pH, ArsS is inactive and ArsR is not phosphorylated. This ArsR binds to the promoter of the sRNA that targets the *ureB* part of the *ureAB* mRNA (ureB-sRNA) and consequent truncation of *ureAB* mRNA with a decline in urease activity. This reflects a likely adaptation to neutral pH. At acidic pH, ArsS is activated with ArsR phosphorylation and this results in upregulation of *ureAB* mRNA and consequent increase in acid-protective urease activity

hence untreatable at intragastric pH < 3.0 [44] contributing to the >30 % failure of standard triple therapy in acid hypersecretors. Some adverse effects of PPIs include hypocalcaemia in elderly patients undergoing chronic therapy [45]. A very small number of patients may develop hypomagnesaemia with serious effects on the central nervous, cardiac, and musculoskeletal systems [46], possibly due to a mutation in the duodenal Mg transporters enhancing their susceptibility to activated PPIs in the duodenum [47]. On the whole, however, given their relatively minor adverse effects discovered only after millions of patient-years of experience, and their unceasing and potent effectiveness, which has revolutionized the treatment of acid-peptic foregut disease, these drugs have a remarkably low risk/benefit.

Reversible Inhibitors of the Gastric H^+, K^+ ATPase

With generation of the E_2P form, a luminal vestibule is formed by changes in orientation of the transmembrane domains (illustrated in Fig. 14), emphasizing the access pathway for inhibitors such PPIs and the K^+ competitive inhibitors of the pump. In the early 1980s, my laboratory was exploring the role of Ca^{2+} in stimulus-secretion coupling in the parietal cell, testing Ca^{2+} channel blockers such as trifluoperazine, verapamil, and 8-(*N*,*N*-diethylamino)octyl-3,4,5-trimethoxybenzoate on acid secretion in rabbit gastric glands. The K_i for acid secretion is at least ten times higher than the K_i for Ca^{2+} channel blockade, due to direct competitive inhibition of the ATPase with K^+ [48]. Based on this study, we demonstrated that an experimental antisecretory drug SCH28080, originally synthesized as an omeprazole mimetic, was in fact a K^+ competitive proton pump inhibitor [49]. Due to the huge PPI market, intense pharmaceutical interest has been directed at the discovery of a potent, safe, and long-lasting antisecretory drugs to compete with the PPIs, as illustrated in the Fig. 15, which eventuated in the synthesis of pyrrolo-pyridines such as TAK-438 [50, 51]. The success of this new class depends on very slow dissociation from the pump with binding to the same vestibule as omeprazole. This compound, currently under development in the Far East, if successful will have the advantage of immediate, meal independent inhibition of acid secretion and no need for enteric coating.

Helicobacter Pylori

In 1983, Warren and Marshall provided evidence that infection with *Hp* contributes substantially to duodenal ulcer recurrence [7] (Fig. 16). This discovery revolutionized the concepts of pathogenesis and the treatment of PUD. When the relationship between *Hp* infection and gastric adenocarcinoma was found, it seemed clear that such infection should be actively treated. Nevertheless, there is still controversy as to whether the bacterium is a pathogen or a commensal [52], hence the concept of test and treat is not universally accepted [53]. The relationship of *Hp* infection to PUD and to gastric cancer created a paradigm shift in treatment of PUD [54], suggesting strongly that prophylactic eradication is justified. It is universally accepted that eradication is needed for symptomatic disease.

There are two major areas of current research, the host response and the mechanisms behind the ability of the organism to colonize the human stomach. In my laboratory, we have been mostly concerned with discovering the means whereby only *Hp* is able to colonize the normal human stomach. *Hp* is a neutralophile, meaning that it grows best at neutral pH and does not grow at pH < 5.0 or >8.2. The key property exhibited by this organism is its ability to buffer its periplasm to near neutral in acidic environments, mimicking a neutralophilic environment (Fig. 17). The transcriptome of *Hp* recovered from the gerbil stomach is consistent with a <pH 4.0 habitat [55].

Eradication of *Hp*

In the 1990s, triple therapy using two antibiotics such as amoxicillin with either clarithromycin or metronidazole and an antisecretory drug such as an H_2RA or PPI was an effective means of *Hp* eradication, with ~90 % efficacy. In the twenty-first century, resistance to the latter two antibiotics reduced efficacy to <70 %, requiring a different regimen [53]. To improve eradication rates, which is of particular importance given the billions of infected individuals worldwide, either novel antibiotic independent targets are required or a modification of antibiotic therapy is required. One novel method is to add bismuth subcitrate to the triple therapy, termed quadruple therapy, or to add other antibiotics, although these approaches only modestly improve eradication rates [56]. From the analysis of the gastric biology of the organism, several druggable targets have been discovered, such as the external facing proteins UreI, HP0165, or FrbP4 or ExbD or NixA [57, 58]. Interference with these molecular targets will require development of novel agents present in the stomach for a sufficient time to facilitate *Hp* killing.

Another approach derives from an understanding of the need for acid suppression coupled with antibiotics in eradication regimens. As noted above, at pH 3.0 the organism ceases to grow, developing a persistent phenotype. Potent acid suppression, achieving pH > 5.0, 24 h/day, abolishes this persistence [44]. Although current PPIs fall short of this goal, the use of a more effective PPIs such

as the prodrug of omeprazole, AGN904, or the aforementioned long-acting K^+ competitive inhibitor, TAK-438, may overcome this problem. Since resistance to amoxicillin is very rare, treatment with omeprazole and amoxicillin in slow omeprazole metabolizers which suppresses acid secretion to near this level resulted in excellent eradication [59], supporting this strategy. Hence, this dual therapy could successfully replace current triple therapy.

Summary

In the last quarter of the twentieth century, the treatment of PUD radically changed from the former mainstays of diet and surgery to the development of H_2 receptor antagonists that were the first effective medical means of PUD treatment. Nevertheless, a relatively weak response for heartburn or gastroesophageal reflux disease and tolerance development prompted the search for more effective treatments. The discovery of the gastric H^+, K^+ ATPase, the final step of acid secretion, termed the proton pump, followed by the development of the proton pump inhibitors forever altered PUD and GERD treatment. The discovery of the causative role of infection by *Hp* now make its eradication the standard-of-care for patients with gastric symptoms. All clinically useful *Hp* eradication regimens include a PPI, presumably to reduce the persistent state of the organism in the stomach by elevating intragastric pH. The future may lie in application of more effective means of eradication and more effective inhibitors of the gastric proton pump.

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