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The Gastric H,K-ATPase as a Drug Target

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Introduction

Gastric acid is secreted by parietal cells in the stomach. These have two known acid stimulatory receptors the H_2-receptor and the muscarinic M_3 receptor. Gastrin, the major endocrine activator of acid secretion, exerts its action via release of histamine from the ECL cell as does pituitary adenylate cyclase activating peptide (PACAP), a neural mediator of activation of acid secretion. Antagonists of the former two stimulants inhibit gastric acid secretion. Cholinergic receptor antagonists have many side effects and are relatively weak inhibitors at therapeutic doses as compared toH_2-receptor antagonists. These drugs were widely developed in the 1970's and 1980's and became the first really useful medications for healing of peptic ulcers. However, although good for healing peptic ulcers, they were less effective in treatment of erosive esophagitis. Moreover, tolerance was found reducing their efficacy by ~50% after ~ 5-7 days of treatment. They were effective in suppressing night time acidity but less so against day time acidity.

A new target, the gastric H,K-ATPase, the transporter catalyzing the final step of acid secretion, supplanted the H_2 receptor antagonists in the 1990's. The first class of drug to be used clinically is the proton pump inhibitor class. Proton pump inhibitors covalently bind to the gastric H,K-ATPase under acidic condition and inhibit the enzyme activity. A second class of drug is under developments defined as acid pump antagonists (APAs) that inhibit enzyme activity by competing with the K^+ on the luminal surface preventing turnover of the enzyme. In this review, the structure and function of the gastric H,K-ATPase will be discussed first. Then the inhibitory mechanism of proton pump inhibitors and acid pump antagonists and pharmacodynamics of these drugs will be discussed.

Function of the gastric H,K-ATPase

The gastric H,K-ATPase in the parietal cell of the gastric mucosa is responsible for the transport of HCl through membrane by H for K exchange catalyzed by ATP driven phosphorylation/dephosphorylation [1]. It catalyzes an electroneutral exchange of cytoplasmic protons for extracytoplasmic potassium. In the resting parietal cell, the pump enzyme is present in smooth surfaced cytoplasmic membrane tubules. Upon stimulation of acid secretion, the pump is now on the microvilli of the secretory canaliculus of the

parietal cell. This morphological change results in a several fold expansion of this structure [2]. In addition to this transition, there is activation of a K and perhaps a Cl conductance in the pump membrane which allows K to access the extra-cytoplasmic face of the pump, enabling dephosphorylation and recycling of the pump [3].

The H,K-ATPase catalyzes transport by means of conformational changes driven by cyclic phosphorylation and dephosphorylation of the catalytic subunit of the ATPase, The H,K-ATPase binds hydronium ion. With phosphorylation, the conformation changes from $E_1P{\bullet}H_3O^+$ to $E_2P{\bullet}H_30^+$ form. After releasing H_3O^+ and binding of K^+ on the extra-cytoplasmic surface of the enzyme, the $E_2P{\bullet}K^+$ conformation is formed. $E_2P{\bullet}K^+$ conformation then converts to E_1K^+ conformation following dephosphorylation. E_1K^+ conformation releases K^+ to the cytoplasmic side, allowing the rebinding of H_3O^+ and completing the enzyme cycle as shown in figure 1.

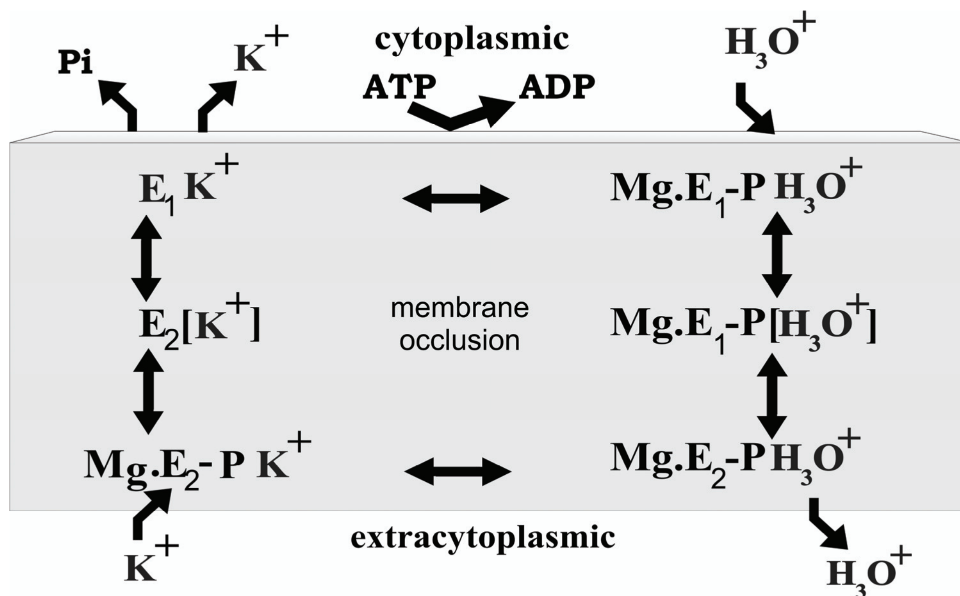

Figure 1. Enzyme reaction cycle of the gastric H,K-ATPase

The enzyme binds MgATP on the cytoplasmic surface in the nucleotide binding domain and along with binding of the hydronium ion in the ion binding site, the catalytic subunit of the acid pump is phosphorylated to form the E_1P conformation with the ion binding site accessible from the cytoplasmic surface and the enzyme is still able to phosphorylate ADP. The ion bound is then enclosed within the membrane domain to form the occluded state, which then opens to the outside surface to form the E_2P conformation and release

acid to the outside surface of the pump. K then binds from this surface, resulting in dephosphorylation and formation of the occluded E_2K form that then converts to E_1K enabling release of K to the cytoplasm upon binding of ATP and restarting the cycle

3D Structure of the gastric H,K ATPase

There are several mammalian P_2-type ATPases such as Ca-ATPase, Na,K-ATPase, and the gastric and colonic H,K-ATPases. Although each of these ATPases enables specific transport of a particular ion, there are significant structural homologies in these three P-type ATPases. The ATP binding domain, phosphorylation domain, and ion binding domain in particular share amino acid sequences. The hydropathy profile of these ATPases is also very similar, as is the 3D structure as defined by the effects of site directed mutagenesis [4-6]. All these ATPases utilize the ion binding properties of several carboxylic amino acids in their membrane domain to bind their transported cations.

In spite of these similarities, there is lack of homology in other regions enabling ion selectivity and allowing for specific inhibitors for each type of ATPase. For example, the cardio-glycosides such as digoxin have only one known target, the Na,K-ATPase. Similarly, thapsigargin targets the sarcoplasmic and endoplasmic reticular but not the plasma membrane Ca-ATPase. There is about 60% sequence homology between the Na,K-ATPase and the H,K-ATPase subunit [7], while the Ca-ATPase of sarcoplasmic reticulum shows only about 29% overall homology with the H,K-ATPase.. In contrast to the ER or plasma membrane Ca-ATPases, the Na,K- and H,K-ATPases are assembled with a β subunit. The H,K-ATPase subunit has 35% homology to the β_2 subunit of the Na,K-ATPase [8].

The gastric H,K-ATPase consists of an -subunit of about 1034 amino acids [9] and -subunit glycoprotein having about 290 amino acids [10, 11]. The H,K-ATPase -subunit has ten transmembrane segments and -subunit one transmembrane segment. The H,K-ATPase -subunit has a strong association with the -subunit. The luminal loop between the seventh transmembrane segment (M7) and the eighth transmembrane

segment (M8) of the subunit is one region associated with the subunit [12, 13]. The H,K-ATPase exists as an ( )$_2$ heterodimeric dimer. The cytoplasmic loop containing the N domain between the fourth and the fifth transmembrane segments appears to have close proximity between two subunits as shown by cross-linking [14]. However there are not adequate resolution crystals of either the Na,K or H,K-ATPase to define the regions of the α or β subunits that are interacting.

The primary sequences of the subunits deduced from cDNA have been reported for pig [9], rat [15] and rabbit [16], dog [17] and man [7]. The hog gastric H,K-ATPase subunit sequence deduced from its cDNA consists of 1034 amino acids and has a Mr of 114,285 [9]. The sequence based on the known N-terminal amino acid sequence is one less than the cDNA derived sequence [18]. The rat gastric H,K-ATPase consists of 1033 amino acids and has a Mr of 114,012 [15], and the rabbit gastric H,K-ATPase consists of 1035 amino acids, with a Mr of 114,201 [16]. The degree of conservation among the subunits is extremely high (over 97% identity). The putative distal colon H,K-ATPase subunit has also been sequenced and shares 75% homology with both the H,K and Na,K ATPases [19]. This enzyme can also transport Na relatively efficiently

The sequences of the subunits have been reported for rabbit [11], and hog [20]. There is one membrane spanning region predicted by the hydropathy analysis, which is located at the region between positions 38 and 63 near the N-terminus. There are 6 or 7 glycosylation sites dependent on species that are important for targeting this subunit to the apical membrane in polarized cells [21].

The first crystal structure of the sarcoplasmic reticulum Ca-ATPase resolved at 2.6Å resolution shows two calcium ions bound in the transmembrane domain that consists of ten alpha-helices [22] and is the E_1 form. Several other forms have been crystallized such as the E_2.thapsigargin, the E_1.ATP and the E_2P states [23, 24]. These structures allowed visualization of essential conformational changes during the transport cycle of this calcium/proton transporting enzyme. In the Ca-ATPase, the N-domain where ATP binds then inclines nearly 90° with respect to the membrane and the A domain rotates by about 110° horizontally during the change from the E_1 to the E_2 conformation. There are also conformational changes in the membrane domain induced by motion of M1 in the

actuator domain. In the absence of Na,K-ATPase or H,K-ATPase high resolution crystals, several methods have been used in order to relate the structure of these ATPases to the structure of sr Ca-ATPase such as site-directed mutagenesis, cleavage patterns of different conformations and molecular modeling [25-27].

A detailed homology model has been published recently identifying the mechanism of outward proton transport and the site of K reentry that also was related to the results of site directed mutagenesis [28]. The key conclusions from this approach were that the exported hydronium at acidic pH is liganded by Asp824, Glu 820 and 795 and is displaced outward by insertion of Lys 791 into this region. The K binding region is predicted to be enclosed by carbonyl oxygens in the non-helical part of TM4 and Glu 795 and 820. K binding moves these away from Lys 791 allowing return of this side chain and then K occupies the site formerly bound by H_3O^+ and the pump dephosphorylates with return of K to the cytoplasm. A highly simplified schematic of these steps is shown in figure 2.

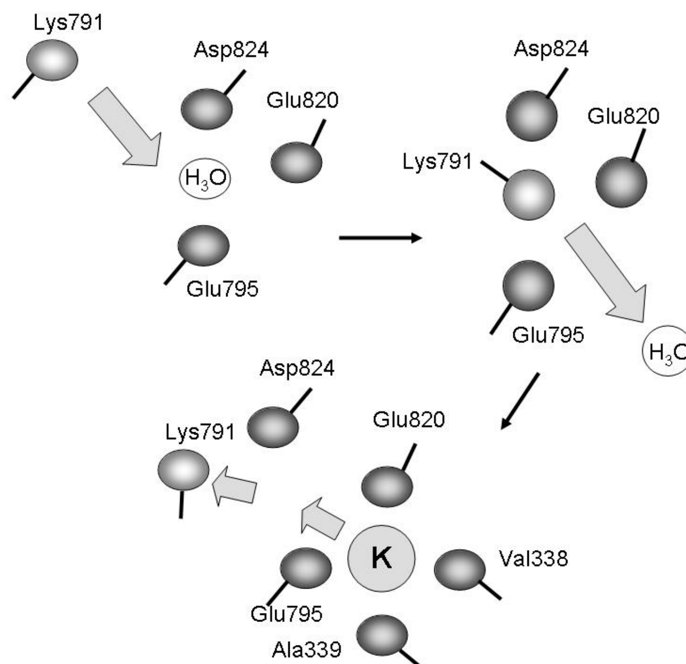

Figure 2. *A model of the mechanism of outward proton transport and the site of K reentry. Upper left shows the hydronium bound between glu795 (TM5) and glu820 and asp 824 (TM6) with the lys791 in TM5 poised above the ion binding region. With phosphorylation, lys791 rotates into the hydronium site, displacing the cation because of*

the higher affinity of the –NH_3^+ of the lysine, upper right. K binds between val338 and ala339 (TM4) and glu 795 and glu 820, relaxing the hydrogen bonding of the –NH_3^+ of lys791 allowing return of this side chain towards the E_{occ} conformation, followed by K binding in the ion binding domain. K then returns to the cytoplasmic side, restoring the E_1K conformation that is then moved into E_1 by the binding of MgATP.

Inhibition of the gastric H,K-ATPase

Since H^+ transport by the gastric ATPase is the final step in acid secretion, the inhibition of the gastric ATPase would be an effective means of regulating acid secretion and cannot be surmounted by alternative stimulatory pathways as is the case with receptor antagonists. There are various parameters that need to be considered when designing a drug for inhibition of this enzyme.

There has to be a large therapeutic index. Acid related diseases are in general not life threatening and although theoretically less effective, there are several H_2 receptor antagonists available both as generics and over the counter in most countries in the world. These latter drugs have a large margin of safety hence compounds that treat acid related diseases by inhibition of the gastric acid pump have to have at least an equal margin of safety. Such a drug must be selective for the gastric H,K-ATPase. The gastric H,K-ATPase has 75% homology to the Na,K-ATPases and the colonic H,K-ATPase. Hence inhibitors of the H,K-ATPase must not interfere with functioning of these ATPases and also should affect as few other targets as possible.

Whereas design of small ligand antagonists is not easy, at least the structure of the natural ligand provides a template for such molecules in the case of receptors. For the H,K-ATPase, the natural ligands are ATP and the hydronium ion, H_3O^+, on the cytoplasmic side and K^+ on the luminal surface [29, 30]. A compound competing with ATP in cell cytoplasm would likely be non-specific, likely affecting other ATPases and perhaps even other ATP dependent processes. Design of molecules to act as H_3O^+ surrogates would have to take advantage of a special structure in the pump able to bind hydronium ion without affecting other such binding sites. Similar considerations would apply to design of compounds substituting for K^+ on the outside surface. Such

compounds could not be designed based on the structure of these small cations and would be much larger and unlikely to be able to occupy the ion binding sites.

Fortunately, an advantage accrues in drug design from the special environment of the functioning ATPase. In the secreting parietal cell, the active pumps are present in the microvilli of the secretory canaliculus and produce a pH of ~0.8, an acidic gradient in this space that is > 1000 fold more acidic than anywhere else in the body. Protonatable weak bases will accumulate selectively in the canalicular space as a function of their pK_a. This is because the protonated positively charged form is significantly less permeable than the uncharged species. Since this is the only space in the body with a pH of less than 4.0, weak bases with a pK_a of 4.0 or less will accumulate only in the canaliculus of active parietal cells since the unprotonated form permeates the basal lateral and canalicular membrane of the parietal cell and the protonated form, being relatively membrane impermeable, will concentrate in the canaliculus. In the fully active cell a compound of a pK_a of 4.0 will accumulate about 1000 fold in this space and gain a significant therapeutic advantage.

The acidity of the space could also confer a chemical advantage if such weak bases are inactive but are also acid labile and convert rapidly in the acidic secretory canaliculus to compounds able to inhibit the ATPase. As will be seen, this prodrug concept has played a vital role in the development of the current drugs used to control acid secretion by covalent pump inhibition.

The proton pump inhibitors

In 1972, CMN 131 (2-(pyridin-2-yl)ethanethioamide) was found to have anti-secretory activity at Hässle AB in Göteborg, Sweden. In 1973, SK&F announced the development of cimetidine, the world's first H_2 receptor antagonist [31]. Based on the structure of cimetidine, a benzimidazole ring was added to CMN 131 and still anti-secretory activity was observed *in vivo*. Finally the sulfide was modified for stabilization to a sulfoxide and timoprazole was born [32].

This compound had rather remarkable anti-secretory properties: it inhibited gastric acid secretion whatever the stimulus; it inhibited secretion in isolated gastric glands whatever the stimulus but was relatively acid unstable and also showed inhibition

of iodide uptake by the thyroid and was thymotoxic. The first polyclonal antibody against the H,K-ATPase reacted with stomach, but also mysteriously with the thyroid and thymus. This suggested that perhaps the ATPase was the target of timoprazole. By 1977, a compound picoprazole had been made retaining the core structure of timoprazole. It was shown that this compound and timoprazole did inhibit the gastric H,K-ATPase but only when the ATPase was making acid, and that there was a lag phase of inhibition of transport activity. Since the compound was a weak base, the steps that were thought then to result in inhibition of ATPase activity and acid secretion involved accumulation of the compound in the acid space of the isolated, intact gastric vesicles during H^+ transport (or the parietal cell canaliculus) followed by acid dependent conversion to an active compound to account for both the acid dependence and lag phase of inhibition [33]. It was postulated that this class of compound acted as a prodrug that only reacted with the ATPase after this acid catalyzed conversion to an active form, perhaps the sulfenic acid or sulfenamide [33, 34]. Later, it was shown that the final structure generated in acidic solutions was a rearranged planar tetracyclic compound containing a highly –SH reactive sulfenamide group [35]. It is not clear whether the sulfenic acid or its dehydro form, the sulfonamide is responsible for covalent binding to the H,K-ATPase. In order to optimize the acid stability of the parent compound and to generate absolute selectivity for accumulation/activation in the acid space of the parietal cell, omeprazole was synthesized in 1979 and became the compound that was launched in 1988 at the Rome World Congress of Gastroenterology.

The name for this class of drug is proton pump inhibitors (PPIs). Following publication in 1981 of the first of a series of papers on the mechanism of action of these drugs [34], a variety of derivatives were synthesized that also led to the introduction of other drugs with generally similar properties to omeprazole [36-43].

The core structure of all currently available benzimidazoles, with points of substitution is shown in Figure 3.

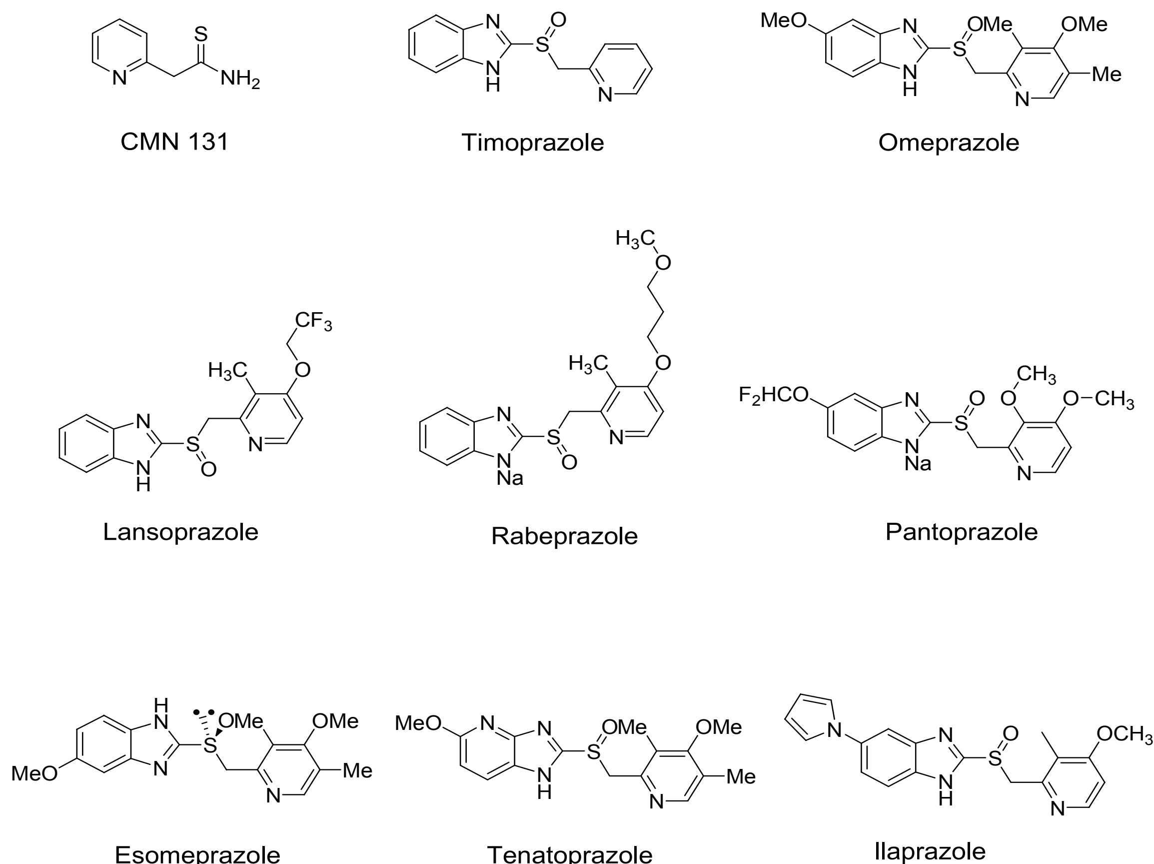

Figure 3. *Proton pump inhibitors and related compounds. On the top left is shown the 2-pyridinyl ethanethioamide, CMN131, that had gastric anti-secretory activity. Next is shown timoprazole where CMN131 was modified by generation of the sulfoxide and addition of the benzimidazole ring. Then a series of compounds based on this core structure is shown, all of which have largely equivalent anti-secretory activity. Of these, tenatoprazole and ilaprazole are undergoing development, the other five are in clinical use.*

Mechanism of action of the PPIs

Chemical mechanism of activation of PPIs

The key steps are (a) acid gradient dependent accumulation of the prodrugs (b) an acid or enzyme surface catalyzed conversion of the prodrugs to a sulfenic acid and thence to a sulfenic acid o tetracyclic, planar sulfenamide. A detailed mechanistic analysis of the chemistry behind the substituted benzimidazoles was published recently [44].

Changes the basic reaction mechanism stated here can be modified by a change in the pK of this group.

The conversion rate of PPIs to the active sulfenic acid or cyclo-sulfenamide is determined by both electrophilicity of C-2 of benzimidazole ring and nucleophilicity of the pyridine moiety fact. The electrophilic attack by the C-2 of the protonated benzimidazole on the nucleophilic pyridine N enables an intramolecular rearrangement generating a C(2)-N(pyridine) bond to form the thiophilic sulfenic acid and after dehydration the cyclo-sulfenamide, both of which are permanent cations and membrane impermeant. These can bind the luminally exposed cysteines of the H,K-ATPase and do not cross the membrane of the secretory canaliculus, the location of the activated ATPase.

The nucleophilicity of pyridine ring depends on the electronic effect of substituents on the pK_a of the pyridine ring. When electron-donating substituents are attached to the pyridine moiety, the pK_a of pyridine increases, thus increasing its protonation at any given pH. Protonation of pyridine ring then obviously decreases its nucleophilic reactivity due to the occupation of the lone pair of electron of the pyridine N by the proton. However, the nucleophilic reactivity of the unprotonated form is increased. Substituents on the benzimidazole influence the electron density at the C-2 position. Although electron-donating substituents will decrease the electrophilicity of C-2 position of benzimidazole, they will raise its pK_a2 hence increasing protonation on N-3 position of benzimidazole at a given pH. The converse is true of electron withdrawing substituents where protonation on N-3 position of benzimidazole will be decreased at a given pH. Protonation of the benzimidazole N-3 position increases the electrophilicity of the C-2 carbon since protonation of the vicinal nitrogen will withdraw electrons from the C-2 position. This will dramatically increase the rate of reaction with the fraction of the pyridine N that is not protonated. In summary, it is the protonation of the imidazole N vicinal to the 2C that determines the reaction rate with the unprotonated pyridine and this pKa ranges from -0.1 for tenatoprazole to 0.1 for pantoprazole, 0.6 for lansoprazole, and 0.8 for omeprazole [44].

Recent findings may suggest that the pump itself may play a role in its own inhibition in that perhaps the sulfenic acid is formed on the surface of the pump and this is the reactive intermediate binding to the relevant cysteines.

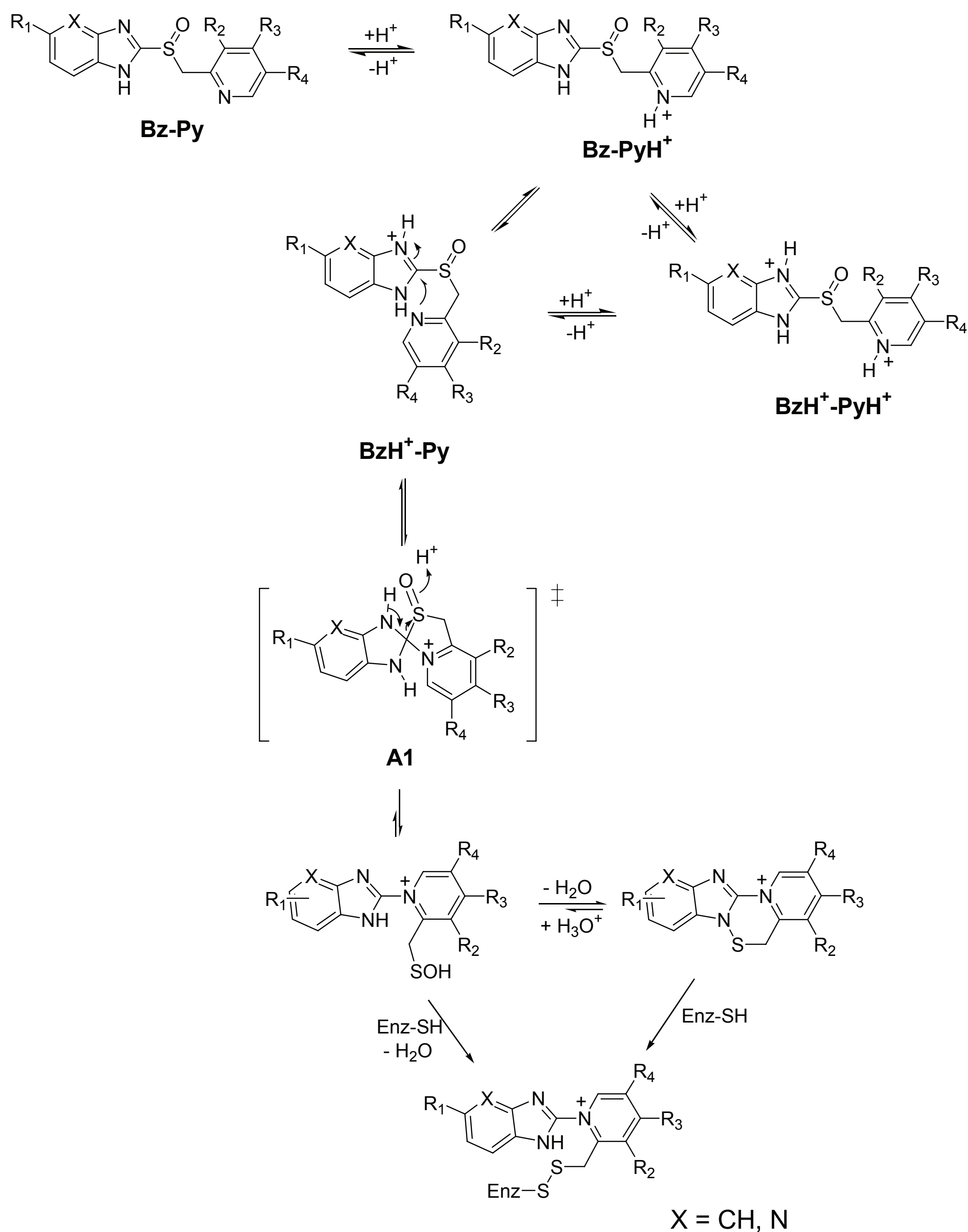

Figure 4. *Mechanism of acid activation of proton pump inhibitors. The first step is protonation of the pyridine nitrogen in all the PPIs, which accounts for the accumulation in the parietal cell. The second protonation is the activation step. In all the PPIs, the activating protonation occurs at the nitrogen vicinal to the C-2 position of the imidazole ring. In the particular case of tenatoprazole where X is N instead of C as in all other*

PPIs, the second protonation is spread throughout the imidazopyridine. This second protonation results in a fraction of the species being present with an activated C-2 position and an unprotonated pyridine, which can then proceed to form the sulfenic acid via a transition state [A1]‡. In solution, this proceeds to form the sulfenamide by dehydration. In the presence of thiols, the sulfenic acid reacts to form the disulfide hence, accelerating the reaction. Either the sulfenic acid or the sulfenamide can react with the luminally accessible cysteines of the gastric H,K- ATPase [44].

Biological mechanism of PPIs

1. Cellular mechanism

Conversion of PPI to an active form requires gastric acid. These active forms, either the cyclic sulfenamide or the sulfenic acid is very reactive with the thiol groups of cysteine which can then form a disulfide bond with inhibition [35].

Whole body autoradiography of a mouse injected intravenously with tritiated omeprazole showed general labeling after 5 minutes, but after some hours, labeling was found only in the stomach [45]. Higher magnification showed that labeling was present only in the parietal cell [46]. This means that not only was there highly targeted accumulation of the drug, but that the binding in the parietal cell was covalent, since it remained for several hours.

Reaction of omeprazole with isolated gastric glands results in inhibition of acid secretion [47]. Treatment with thiol reducing agents (mercaptoethanol, cysteine, glutathione) reversed the inhibition in the parietal cell [48, 49]. Omeprazole therefore depends on formation of disulfide bonds for its inhibitory action on gastric acid secretion as deduced from its chemistry. Both cysteine and glutathione were effective and glutathione depletion increased the efficacy of omeprazole [49].

Treatment of isolated gastric glands with tritiated omeprazole under non-secreting and acid secreting conditions allowed analysis of the distribution of the drug as a function of time. The drug showed labeling mainly under acid secreting conditions and initially labeled only the canalicular compartment. In the unstimulated state there is no labeling of the canaliculus until about 30 min after introduction of omeprazole. This is due to a

recycling of the labeled mump to the resting tubule conformation at the basal level of acid secretion. With stimulation, there is rapid labeling of the canaliculus with a half-life ($t_{1/2}$) of about 10 min. The tubules are not labeled until about 30 min. Hence, omeprazole reacts only with the pump in the membrane of the acidic canaliculus. Labeling of the cytoplasmic tubules depends on retrieval of the pump from the canalicular membrane [46].

The mechanism of inhibition of the gastric H,K-ATPase *in vivo* that results from these experimental data suggests that the drug, provided it is given in a gastric acid protected form, reaches the cytoplasm of the cell via the circulation.

2. Binding sites of PPIs in the gastric H,K-ATPase

Treatment of a rabbit [46] or rat [50] with radioactive omeprazole, isolation of gastric membranes and separation using SDS-Page, showed that only the catalytic subunit of the H,K-ATPase was labeled. The drug is accumulated on the luminal face of the pump where it undergoes acid conversion to the sulfenic acid and the sulfenamide which then reacts with one or more exposed cysteines on the luminal surface of the enzyme.

Reaction of PPIs with the isolated ATPase resulted in inhibition of the ATPase only under acid transporting conditions [51, 52]. This was found for all the PPIs. Inhibition by omeprazole was reversed by mercaptoethanol or dithiothreitol also showing that disulfide formation was responsible for inhibition of the gastric acid pump [49, 50].

The PPIs inhibit ATPase activity or acid transport at different rates *in vitro*. The order of reactivity was rabeprazole > omeprazole = lansoprazole > pantoprazole > tenatoprazole, consistent with the order of acid stability [50, 53, 54]. This is explained by the relatively weak acidification in gastric vesicles allowing a measurable separation of inhibition based on the relative rates of activation of the compounds.

The location of the binding sites of the radioactive PPIs on the pump enzyme under *in vitro* acid transporting conditions identifies the one or more cysteine(s) critical for inhibition of enzyme activity. Labeling at essentially full inhibition showed that lansoprazole labeled cys 321, 813 or 822 and cys 892 [55]. A similar set of cysteines was labeled by rabeprazole. Omeprazole labeled cys 813 or 892 with slight labeling at cysteine 892 [53, 56], whereas pantoprazole labeled both cys 813 and 822 and no other

cysteine [57, 58]. Cys 321 is at the end of TM3, cys 813 and 822 are in the TM5/TM6 domain and cys 892 is in the large outside loop between TM7 and TM8.

When the time course of labeling was compared to the time course of inhibition for all the PPIs, labeling of the cysteines in the TM5/6 region (either cys 813 or 822) correlated with inhibition. Definition of which of these two cysteines was labeled was done by using labeled omeprazole under transport conditions, tryptic digestion, isolation of the labeled peak by SDS-tricine gradient PAGE, re-digestion with thermolysin and sequencing of the peak retaining the label. It was deduced that cys 813 was labeled in the M5/M6 domain [53]. Cys 892 is in the large exocytoplasmic loop between M7 and M8 and does not participate at all in inhibition of acid transport.

3. Reversal of Acid Secretory Inhibition

Measurement of protein turnover in the rat showed that the alpha subunit of the pump had a half-life of 54 hr and that this was unaffected by proton pump inhibition [59]. It was prolonged to about 120 hr, however, by treatment with histamine 2 receptor antagonist (H2RA). PPI treatment does not affect recycling of the pump between cytoplasmic tubules and canalicular membrane microvilli, whereas H2RA's return all the pumps to cytoplasmic tubules, thus abolishing recycling and proteolysis following membrane endocytosis. However, acid secretion and H,K-ATPase activity returned with a half-life of only 15 hr in this species, about three times faster than anticipated if only protein turnover were responsible for recovery of acid secretion. Following treatment of people with PPI's, acid secretion also returns faster than expected from protein turnover for omeprazole or lansoprazole (~20 hr) [60]. Only pantoprazole has a duration of action compatible with recovery due only to pump biosynthesis (~47 hr). Pantoprazole is unique in being able to access both cysteine 813 and 822 and the latter is deeper within the membrane domain as compared to the other cysteines that reaction with the PPI's.

Rats were treated with the different PPIs *in vivo*, namely omeprazole, esomeprazole, lansoprazole, rabeprazole and pantoprazole to provide identical inhibition of acid secretion. The pump was isolated and incubated for different lengths of time with glutathione, the natural reducing agent in the parietal cell. Measurement of the reversal of inhibition of the pump by glutathione showed that inhibition by all the PPIs was reversed

after 60 min incubation, except for inhibition by pantoprazole, where there was no reversal. This is consistent with cysteine 822 being inaccessible to this reducing agent and thus making inhibition by pantoprazole, *exo* or *in vivo* reversible only by *de novo* pump synthesis. This is consistent with the shielded position of cysteine 822 in the membrane domain, in contrast to cysteines 321 and 813. This observation also predicts a longer duration of inhibition by pantoprazole than for other PPIs [50].

Potassium-competitive acid pump antagonists

There are several protonatable tertiary amines that have been synthesized which are capable of K^+ competitively inhibiting the ATPase activity. This class of drug has been called potassium-competitive acid blocker (PCAB) (which was used to be called as acid pump antagonist (APA)). In contrast to the PPIs they are able to inhibit the pump without acid activation, but since they are also weak bases they are accumulated in the parietal cell canaliculus and their potency increased as a function of their pK_a. Further they may bind to a variety of structures since they are not made selective by unusual activation pathways.

The original lead structure, SCH28080 (3-cyanomethyl-2-methyl-8-(phenylmethoxy) imidazo[1,2a]pyridine) binds to the enzyme largely in its protonated form and to a site accessed from the luminal surface of the pump [61]. A photoaffinity analog, $MeDAZIP^+$, after photolysis binds within the membrane domain in the region of TM1 and TM2 [62, 63]. Its K_i, calculating for the protonated form, is in the region of 10 nM. It does not inhibit the Na,K-ATPase, as ouabain does not inhibit the H,K-ATPase.

Derivatives of the SCH 28080 structure, such as BY 841, are also K^+-competitive, and its effect has been studied in people. This compound produces rapid (within 30 min) full inhibition of acid secretion and in principle could be used as a therapeutic antacid with the speed of symptom relief of an antacid and the healing properties of a proton pump inhibitor.

The K^+ competitive acid pump antagonists such as the imidazopyridines bind non-covalently and their specific site of attachment is much harder to predict since the region of the protein that binds K^+ or whose conformation prevents K^+ binding is not known.

Analysis of their mechanism of action showed that the protonated form of an inhibitor such as SCH28080 was more effective and that their reaction was with the external surface of the enzyme [64]. This conclusion was reached since the quaternary form generated by methylation of the pyridine N of SCH 28080 was ineffective in intact right side-out vesicles of the H,K-ATPase and also their K_i decreased with decreasing pH.

Since these inhibitors are non-covalent, the site of binding have to be investigated by mutational analysis or by generating photo-affinity derivatives of, for example, SCH 28080, an imidazo[1,2a] pyridine. The azido form of the methylated imidazo-pyridine, $MeDAZIP^+$, was shown to be K^+-competitive and after photolysis was covalently bound to the TM1/TM2 domain of the H,K-ATPase [63]. Substitution in the loop between TM1 and TM2 did not affect inhibition, resulting in the conclusion that inhibition depends on interaction of the inhibitor with the TM1/2 membrane domain itself rather than with the connecting loop. Construction of a chimer between TM1 and TM2 of the H,K- and the fungal H^+ -ATPase resulted in a SCH28080 sensitive ATPase, again confirming the site of inhibition by these compounds.

A series of mutations has also shown that the loop between M5 and M6 is an important determinant of binding of SCH28080 and has also shown that the binding sites for K^+ and the inhibitor are different and that competitive kinetics arise from mutual exclusion of binding [5, 6].

In contrast to PPIs, the action of these K^+-competitive antagonists is independent of the secretory status and there is no lag time expected since the ATPase is inhibited in the E_2 conformation. These are therefore fast acting compounds able to abolish acid secretion during their presence in the blood. Their efficacy is related entirely to peak plasma levels than area under the curve, unless time release formulations are developed. If these compounds have a plasma half-life similar to that of the PPI's, a pH > 5.0 will be found for about 4 hrs, but acid secretory capacity will be restored fully after two half-lives, namely within 4 to 6 hrs of administration hence a twice a day dosing regimen is required to show superiority over PPIs in terms of 24 hr acid control.

These drugs, if introduced into clinical practice will be therapeutic antacids, allowing on demand dosage, but they will not have the extended inhibitory characteristics

of PPIs. It remains to be seen whether they will achieve the same healing rates of GERD as PPIs although they are predicted to markedly improve the onset of symptom relief.

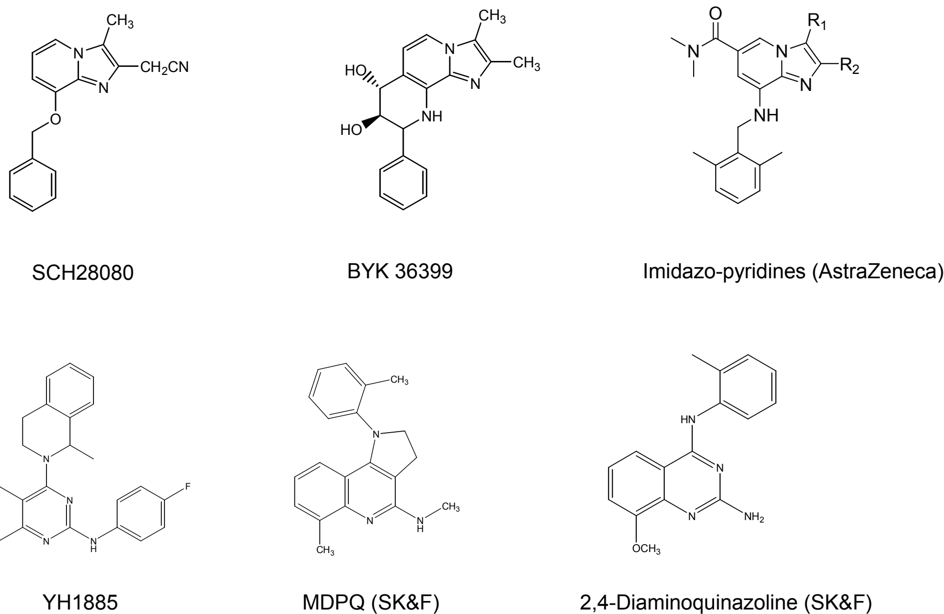

Figure 5. *Potassium-competitive ATPase inhibitors. The first of these is the imidazo[1,2α] pyridine structure, SCH28080 but toxicity prevented its development. The fused ring structure that mimics the active conformation of this core structure is shown as BYK 36399 and another variant of SCH28080 is the AstraZeneca compound that has recently been dropped from further development. A different structure is the Yuhan compound 1885 and other protonatable tertiary amines that were synthesized by SKF are also shown. It appears that the tertiary amine structure is essential for K competitive inhibition of the H,K-ATPase indicated either that the protonated form is the active compound or hydrogen bonding to the unshared electron pair of the N from a residue close to the active site of the H,K-ATPase.*

Inhibitory mechanism of potassium-competitive compounds

One of the typical potassium-competitive inhibitory compounds, SCH 28080 is known to have a higher apparent affinity for E_2P [64] but the stable form appears to be E_2[SCH 28080] [65]. Although SCH 28080 inhibited the ATP phosphorylation at low ATP concentrations, the SCH 28080-bound enzyme was phosphorylated at high

concentration of ATP or phosphate [66]. It was also shown that SCH28080 and other analogs bound only to the outside surface of the E_2P or E_2 form of the enzyme [65, 67].

The region of SCH 28080 binding at the luminal surface of the enzyme consists of transmembrane segments, M4 (Ala^{335}), the M5-6 loop (Leu^{809}), M6 (Cys^{813}), and M8 (Tyr^{922}, Ile^{940}) regions [5, 6]. This is shown in figure 6.

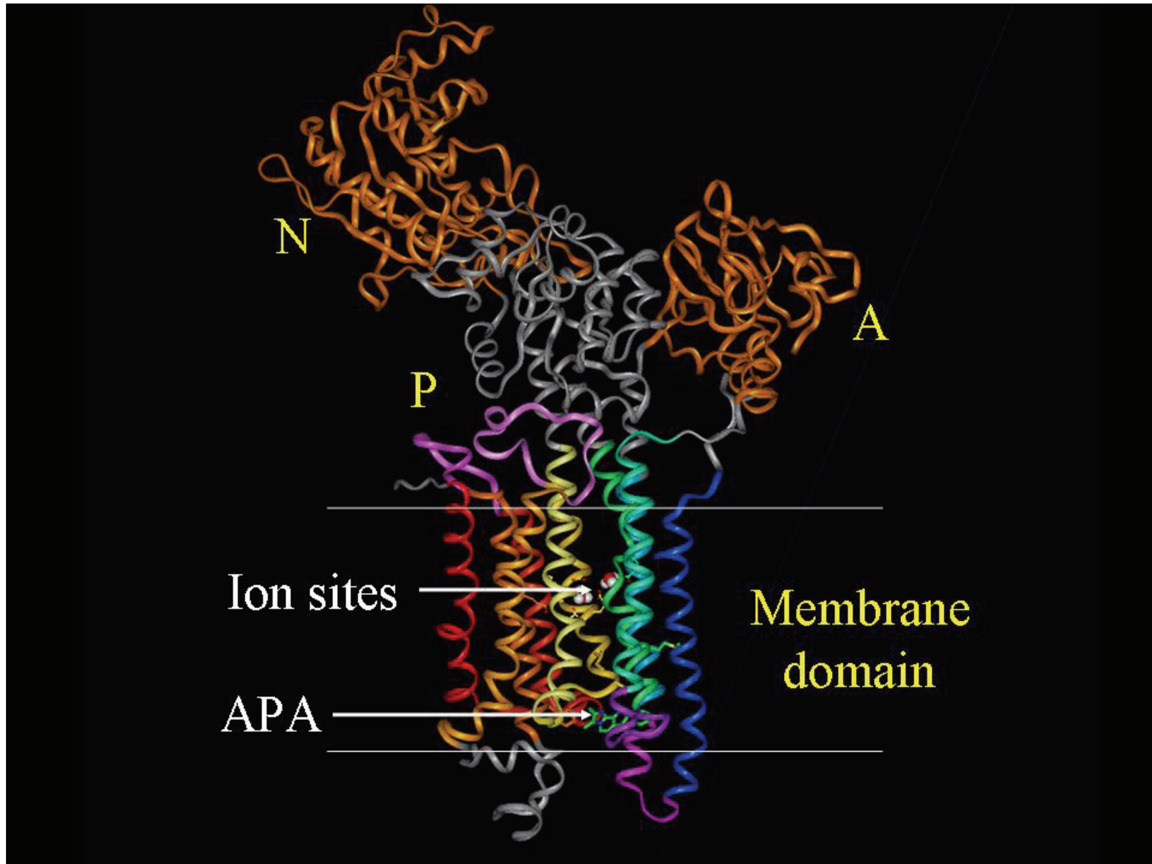

Figure 6: *Homology model of the H,K ATPase showing the N, nucleotide binding, P, phosphorylation and A actuator domain as well as the ion binding sites and the acid pump antagonist bound at the luminal vestibule of the pump in a region overlapping that of the cysteine 813 that reacts with the PPIs.*

Oligomeric structure of the H,K ATPase

2.6 nmol of these drugs bind to 1 mg of the pump enzyme in the presence of MgATP with full inhibition of enzyme activity. The gastric H,K-ATPase has two ATP binding sites with different affinity. About 5.2 nmol ATP bind to 1 mg of the enzyme

with half forming EP and the other remaining bound at a different site unable to transfer its phosphate to Asp386 of the other half of the oligomer. Thus binding at half the ATP sites is sufficient to prevent enzyme turnover. This would be predicted from a dimeric functional oligomeric form of the enzyme [67].

Mg^{2+} binding to the enzyme is stable until the E_1 form is regenerated [64, 68]. Therefore, the E_2•I conformation of inhibitor–bound enzyme appears to chelate Mg^{2+} in the enzyme P-domain in a region inaccessible to CDTA as shown previously for Ca^{2+} binding, in contrast to the Na,K- ATPase [69-71].

The finding that only half of the enzyme formed acid-stable phosphoenzyme while the other half of the enzyme bound ATP without phosphorylation or only half of the enzyme bound inhibitor while the other half did not bind the inhibitor implies that the functional oligomeric enzyme in intact gastric vesicles allows only an E_1:E_2 conformation with two different ATP sites, one with high and one with low affinity [72] that is also reflected by two different Ca^{2+}-binding site affinities, dependent on ATP binding affinity [73]. These inhibitory data for K^+-competitive inhibitor showing a stoichiometry of ~ 2.6 nmol/mg enzyme also correlate with the covalent *in vivo* inhibition of the ATPase with proton pump inhibition with a similar stoichiometry [74].

The benefit of this oligomeric structure of this ATPase is not clear. At the high parietal cell concentrations of ATP, the second half of the oligomer has ATP bound already in the E_1 conformation. This accelerates the formation of E_1H_3O from E_1K at the 150 mM internal K^+ concentration which inhibits enzyme turnover [75] and this half of the oligomer can then proceed rapidly to the transport conformation with the conversion of the ATP bound form to E_1P and then E_2P with proton release, without the delay due to the ATP dependent release of K^+ from the E_1K^+ formed from the other half of the oligomer that has derived from the dephosphorylation of E_2P.

Acid Secretory Inhibition by various drugs

Following the introduction of tagamet in 1977, the medical treatment of peptic ulcer disease was revolutionized. It was given 4 times daily and then with the launch of ranitidine, dosing of the latter was 150 mg twice daily and then famotidine was introduced at 20 mg twice daily. Nowadays, these drugs are often administered at night,

since their effect on diurnal intragastric pH is, for reasons as yet unknown, less impressive. These drugs enabled healing of peptic ulcers within 8 weeks. It was found that in the absence of continued administration, the disease recurred within one year in 60-70% of patients. Their efficacy in healing of GERD, particularly stage III and IV was less impressive[76]. The recurrence of the lesions remained unexplained until the discovery of the causal relationship with infection by *Helicobacter pylori* and the realization that antisecretory therapy of infected patients with peptic ulcer disease must be accompanied by eradication of the organism to reduce the recurrence rate to about 6% [77, 78].

With the development of proton pump inhibitors to treat GERD, a more effective class of drug became available and gradually replaced H_2 receptor antagonists as the treatment of choice for GERD, with equivalent efficacy in treatment of peptic ulcer disease. A major difference was their ability to elevate diurnal pH as compared to the receptor antagonists. [79] Since they are covalent inhibitors of the gastric acid pump and require active parietal cells for efficacy, they are generally prescribed as once a day therapy 30-60 min after breakfast. However, often they are given twice a day initially adding a dose after the evening meal. The available PPIs are omeprazole, lansoprazole, pantoprazole rabeprazole and S-omeprazole. These drugs have very similar profiles in GERD treatment results, although each may have subtle differences. For example, esomeprazole has a longer plasma half life as compared to omeprazole, perhaps due to auto inhibition of metabolism, lansoprazole and rabeprazole may have a faster onset of action and pantoprazole a longer duration of action.

Their mechanism of action, requiring acid activation and thus inhibiting only active pumps, explains the usual finding that therapeutic results are optimal after about three days dosing once a day and that their effect at night is less impressive than their effect during the day [80]. This has resulted in a suggested treatment of a PPI in the morning and an H_2 receptor antagonist at night for treatment of patients who continue to suffer night time GERD on PPI therapy [81].

Eradication of *Helicobacter pylori*

With the recognition of its role in peptic ulcer disease, it became apparent that

eradication of the infection was an essential component of treatment of non-NSAID related peptic ulcer disease. Eventually, the eradication modalities became either a PPI with two antibiotics e.g. amoxicillin, clarithromycin or metronidazole for 7-14 days, bid or ranitidine subcitrate with again two antibiotics [82, 83]. The rationale for combining acid suppression with growth dependent antibiotics is likely due to the fact that the organism in the stomach is often in stationary phase and elevation of the pH of its environment results in a large fraction of the organisms being present in log phase.

Although there is an opinion that *H. pylori* is a commensal, perhaps protecting against GERD due to its ability to neutralize acid by its production of NH_3 and CO_2 from gastric juice urea in an acidic environment [84], most studies show that eradication does not affect GERD. Moreover, its role in gastric cancer suggests that a test and treat strategy is to be preferred. [78]

Future Therapies

It seems fair to say that the current mainstay of therapy, the PPIs, achieves excellent healing within 8 weeks but fall short in terms of symptom relief. In order to improve the outcome of PPI therapy, it is important to prolong the plasma dwell time of such a drug. Two PPIs with a long dwell time are in development, tenatoprazole [85] and ilaprazole [86] and it remains to be seen if their efficacy in symptom relief and GERD healing is improved. Another possibility is long acting H_2 receptor antagonist, lafutidine. [87]

New treatments may involve the use of acid pump antagonists that clearly are more effective in pH elevation but given that these are reversible and not covalent inhibitors, more frequent dosing will be required to show true superiority. Other concepts that are under development are gastrin receptor antagonists [88] and perhaps inhibition of the K channel essential for activation of the gastric H,K-ATPase in the microvilli of the secretory canaliculus [89]. For eradication of *H. pylori*, if specific monotherapy were available, perhaps the test and treat strategy would be universally adopted to avoid risks of peptic ulcer disease and gastric cancer.

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