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THE PHARMACOLOGY OF THE GASTRIC ACID PUMP: The H^+,K^+ ATPase[1,2]

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ABSTRACT

The gastric H^+,K^+ ATPase—the gastric acid pump—is the molecular target for the class of antisecretory drugs called the proton-pump inhibitors (PPIs). These compounds—omeprazole, lansoprazole, and pantoprazole—contain, as their core structure, 2-pyridyl methylsulfinyl benzimidazole. The H^+,K^+ ATPase is a heterodimer composed of a 1034–amino acid catalytic α peptide and a glycosylated 291–amino acid β subunit. The α subunit probably contains 10 membrane-spanning sequences; the β, a single transmembrane segment. The PPIs have a pK_a of about 4.0; hence they accumulate only in the acidic secretory canaliculus of the stimulated parietal cell. Here they undergo conversion to a cationic sulfenamide, which then reacts with available cysteines on the extracytoplasmic face of the α subunit. Omeprazole reacts and forms disulfide bonds with cys813(822) and cys892; lansoprazole, with cys813(822), cys892,

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and cys321; and pantoprazole, with cys813 and -822. The antisecretory effect of the drugs reflects their short plasma half-life (~ 60 min), the number of active pumps during that time, and the recovery of pumps following biosynthesis and reversal of inhibition. These drugs also show synergism with either amoxicillin or clari- thromycin in eradicating *Helicobacter pylori,* an organism shown to be important in duodenal and gastric ulcer disease. Their action is probably due to elevation of pH in the environment of the organism, rather than to any direct action.

INTRODUCTION

Acid secretion by the gastric mucosa is a property of the parietal cell. Whereas the functional regulation of this cell is a complicated process involving several different cell types with several different receptors, acid transport per se is the property of a single P-type ATPase, the gastric H^+,K^+ ATPase. Therefore, effective therapeutic control of acid secretion involves either receptor blockade or H^+,K^+ ATPase inhibition. Most of this review focuses on proton-pump inhibition. References are selective rather than exhaustive because of space limitations.

ACID-RELATED DISEASE AND THERAPEUTIC CONTROL OF ACID SECRETION

At least two factors are required for the development of acid-related diseases. In the case of duodenal and noniatrogenic gastric ulcer, acid and *Helicobacter pylori* infection must be present; in the case of esophagitis, lower esophageal sphincter incompetence and acid; and in the case of drug-induced ulcer, a nonsteroidal antiinflammatory drug and acid. Stress ulcers and steroid-induced ulcers also require the presence of acid. Therapeutic control of acid secretion is therefore fundamental in all these diseases.

A series of meta-analyses have been performed to determine the degree and duration of acid secretory inhibition (as monitored by intragastric pH) necessary for optimal healing of the various acid-related diseases. For treatment of duodenal ulcer disease the pH has to be elevated to a value greater than 3.0 for about 16–18 h per day, from a mean diurnal pH of 1.4 (1). For treatment of esophagitis, the desired pH is also 4.0 for 16–18 h per day (2). For eradication of *H. pylori* with a single antibiotic, the pH apparently has to be elevated to 5.0 for most of the day (3).

Given the complicated pathways of regulation and the short half-life of currently used histamine-2 (H2) receptor antagonists, these intragastric pH changes are difficult to achieve with this class of drug with administration once or twice a day at acceptable doses. This is the main reason for the continuing development of drugs that inhibit the gastric H^+,K^+ ATPase.

REGULATION OF ACID SECRETION

Acid secretion is regulated by intricate central and peripheral mechanisms. Central stimulation relayed by the vagus nerve results in increased parietal cell–acid output. Efferent vagal fibers synapse with ganglion cells of the enteric nervous system, which in turn regulate the activity of three key cell types. Further, acetylcholine liberated from postganglionic nerve fibers directly stimulates the parietal cell (4).

The G-, or gastrin-containing, cell, located in the antrum, is stimulated by food in the stomach or by neuronal input to release gastrin. Gastrin is carried by the blood to the enterochromaffin-like (ECL) cell, where it stimulates the release of histamine by binding to a cholecystokinin-B (CCK-B) receptor (5). This histamine acts as a paracrine agent to activate the H2 receptor on the parietal cell. The ECL cell also responds to epinephrine and acetylcholine with release of histamine. There is also a gastrin receptor on the parietal cell, but apparently this receptor requires some elevation of cAMP for activity as a stimulant of acid secretion (6).

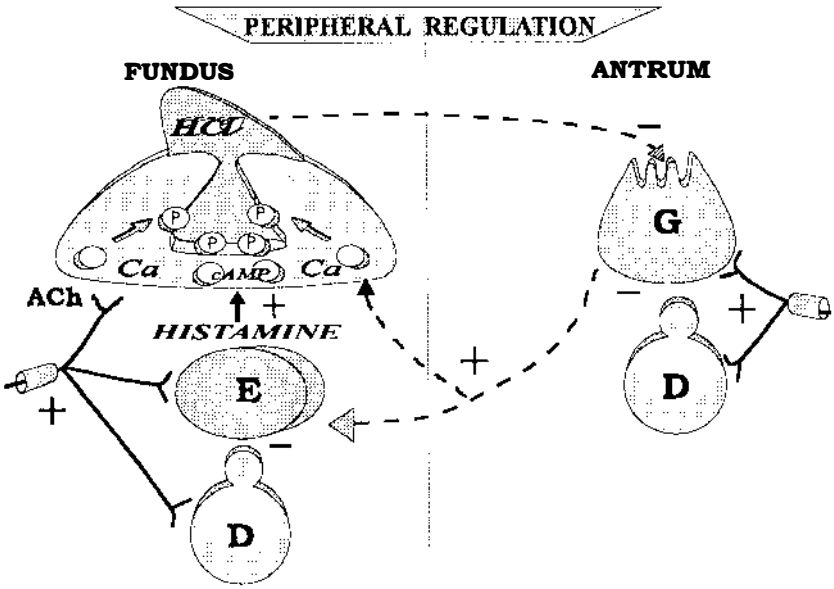

Figure 1 An illustration of the peripheral mechanisms involved in regulation of acid secretion by the parietal cell. On the right-hand side we illustrate the antral G cell and D cells; on the left, the fundic D cell, the ECL cell, and the parietal cell. The D cells release somatostatin that inhibits either G-cell gastrin release or ECL-cell histamine release. The ECL cell releases histamine that stimulates the parietal cell. In addition the neural system releases peptides and transmitters that stimulate the cells illustrated in this diagram.

The D cell or the somatostatin cell of fundus and antrum inhibits either ECL-cell or G-cell function, respectively, by releasing somatostatin (4). Calcitonin gene-related peptide (CGRP) and gastrin induce the release of somatostatin. The acid in the antrum may result in somatostatin release, which then inhibits gastrin release from the G cell (4). This is the major feedback mechanism that prevents excessive acid secretion. In the case of the ECL cell, somatostatin binds to a somatostatin 2 subtype of the receptor and inhibits the histamine release, Ca^{2+} signaling, and histidine decarboxylase activation that result from gastrin stimulation (7). A model for peripheral regulation of acid secretion is shown in Figure 1, where histamine, gastrin, and acetylcholine all bind to the parietal cell. Histamine elevates cAMP and Ca^{2+} in the parietal cell, whereas acetylcholine and gastrin elevate only calcium in the parietal cell. Permissive interactions may occur between the different receptors so that very high doses of single antagonists have pleiotropic effects on parietal cell function.

Therefore, one can interfere with stimulation of the parietal cell at multiple sites, but interference with only one site may not be sufficient to effectively control acid secretion. To reach target pH for treatment of peptic ulcer disease, for esophagitis, and for eradication of *H. pylori,* it is necessary to virtually abolish acid secretion for a large part of the day. To achieve this target, the final step of acid secretion, the H^+,K^+ ATPase must be inhibited.

THE GASTRIC H^+,K^+ ATPase

The H^+,K^+ ATPase is responsible for the elaboration of HCl, when it is present in the canalicular membrane of the parietal cell, where it is associated with a K^+ and Cl^- conductance (8, 9). KCl effluxes from the cytoplasm of the cell, and external K^+ is exchanged for cytoplasmic H^+ at the expense of ATP breakdown. The enzyme, when present in the canalicular membrane, actively secretes HCl. When present in the cytoplasm as part of a tubule membrane that is separate from the canaliculus, the enzyme is inactive and does not elaborate HCl (10), since there is inadequate K^+ access to the luminal face of the pump (8, 9). Stimulation of the parietal cell therefore involves activation of the ATPase by luminal K^+ that is coincident with relocation of the enzymes from the cytoplasmic membrane compartment to the canalicular membrane compartment (Figure 2).

Structure of the H^+,K^+ ATPase

GENERAL This ion pump is a member of the ion-transporting, P-type ATPase family, or the ion-motive-phosphorylating ATPase family. This family extends from bacteria to mammals. The classification depends on the finding that ion transport is coupled to a cycle of phosphorylation and dephosphorylation of

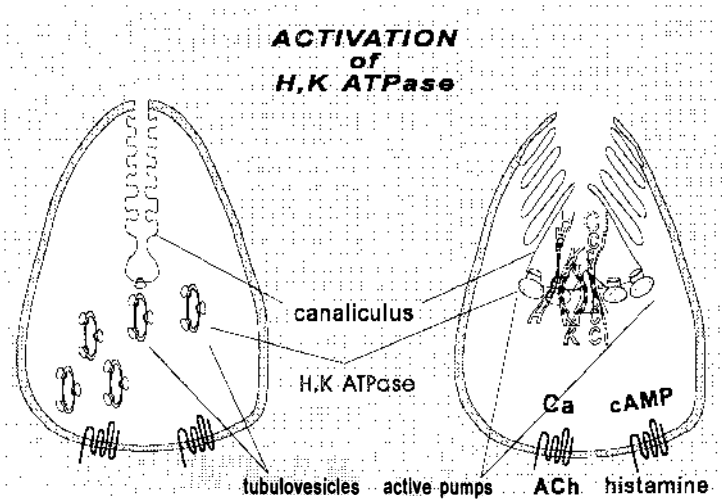

Figure 2 An illustration of the morphological transition and activation of the gastric H^+,K^+ ATPase. (*left*) A resting cell is shown, with a collapsed secretory canaliculus; most of the pumps are tubules in the cytoplasm. (*right*) Following stimulation by either cAMP or elevation of $[Ca^{2+}]_i$, the pumps are found in the membrane of the secretory canaliculus and its microvilli, which are associated with a KCl efflux pathway supplying K^+ to the luminal surface of the pump.

the enzyme. This class of pump is represented in mammalian cells by the sarcoplasmic and endoplasmic reticulum (SERCA) family of Ca^{2+} ATPase (11), the plasma membrane isoforms of the Ca^{2+} ATPase (12), the three isoforms of the Na^+,K^+ ATPase (13), the colonic H^+,K^+ ATPase (14), and of course, the gastric H^+,K^+ ATPase (15–17).

As with the Na^+,K^+ ATPase, the gastric H^+,K^+ ATPase is composed of two subunits. The larger, or catalytic, subunit consists of about 1000 amino acids and is responsible for the transport and catalytic functions of the enzyme (15–17). The smaller β subunit is composed of about 300 amino acids (18, 19), is N-glycosylated, and has a structural and membrane-targeting function.

Whereas the tertiary structure of these enzymes remains mysterious, considerable information has been obtained with respect to their primary and secondary structure. The pumps have a relatively large cytoplasmic domain, a membrane domain, and a small extracytoplasmic domain (20, 21). The membrane and extracytoplasmic domains are relevant to the mechanism and design of acid pump inhibitors.

SECONDARY STRUCTURE OF THE MEMBRANE DOMAIN OF THE α SUBUNIT Defining the membrane domain of these pumps has been quite difficult. Several

methods have been applied, since no single method appears sufficient. The most common approach, hydropathy analysis, predicts seven or nine membrane-spanning segments for the H^+,K^+ ATPase. Ten membrane segments are predicted for the Ca^{2+} ATPase of sarcoplasmic reticulum (sr) by hydropathy (11). Both the N-terminal and C-terminal residues of the majority of the mammalian P-type ATPases are cytoplasmic, based on tryptic cleavage, sided iodination of the C-terminal pair of tyrosines, or epitope mapping (22, 23). Thus, there must be an even number of membrane spanning segments. This shows that hydropathy analysis is incomplete for the H^+,K^+ ATPase.

The catalytic or α subunit of the H^+,K^+ ATPase appears to have 10 membrane-spanning segments, as defined by a combination of tryptic cleavage of inside-out vesicles (24) and by in vitro translation of plasmids that contain putative membrane-spanning segments (25). Inspection of the amino acids present in the membrane-spanning domains, as illustrated in Figure 3, provides some insight into the possible transmembrane ion pathway. Since the H,K ATPase transports H^+ and K^+, it seems likely that the transmembrane ion pathway may contain hydrophilic amino acids. In particular the fifth, sixth, and seventh transmembrane segments appear to have significant hydrophilic character, as illustrated in Figure 3.

Mutation of hydrophilic residues in the sarcoplasmic reticular Ca^{2+} ATPase and the Na^+,K^+ ATPase has shown that the carboxylic acid residues in transmembrane segments M4, M5, and M6 play a role in either catalysis or transport (26, 27). A compound that binds in this region would then be likely to inhibit transport.

Since inhibitors designed for control of acid secretion bind to the extracytoplasmic surface of the pump, the amino acids present in this domain are particularly relevant. The first extracytoplasmic loop contains several carboxylic acids, perhaps a target for the positively charged K^+ competitive inhibitors. The loop between M5 and M6 contains a cysteine, cys813, that may be relevant for inhibition by the 2-pyridyl methylsulfinyl benzimidazoles that are acid-activated thiol reagents. Other cysteines to be considered in this context are cys321 in the M3-M4 region and cys892 in the M7-M8 loop.

MODELING OF THE MEMBRANE DOMAIN In order to create a more detailed structure of the membrane domain than the two-dimensional representation in Figure 3, one must obtain adequate crystals of at least one of the pumps, such as the sr Ca ATPase. The best resolved crystal obtained thus far has been for this Ca^{2+} ATPase. Here the cytoplasmic domain has the appearance of the head of a bird connected by a stalk to a central membrane-spanning core, and a pair of membrane segments appears to be placed on the outside of the central core, perhaps M7 and M8 (21). The extracytoplasmic domain is small.

In the absence of high-resolution crystals, one approach to creating this

Figure 3 A two-dimensional representation of the membrane and extracytoplasmic domains of the H^+,K^+ ATPase, with a single membrane-spanning segment for the β subunit and 10 membrane-spanning segments for the α subunit. This model is derived from (*a*) tryptic digestion of intact, right-side-out membrane vesicles, (*b*) labeling studies with extracytoplasmic reagents, and (*c*) in vitro translation in the presence of microsomes of putative membrane-spanning segments. The rectangles are glutamine; triangles, carboxylic acids; ellipses, hydrophilic amino acids.

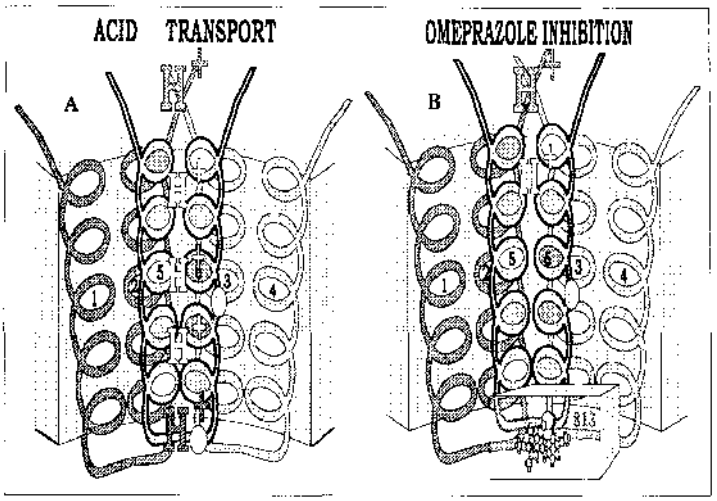

Figure 4 (*A*) A model of the first six membrane-spanning segments of the α subunit, highlighting the fifth and sixth membrane segments and hypothesizing H^+ transport across these segments. Hydrophilic residues in these segments are highlighted. (*B*) A model showing binding of the cationic, acid-activated pyridyl methylsulfinyl benzimidazole to cys813, which inhibits proton transport. Cys822 is less likely based on modeling.

structure is to use a multiple-alignment approach to analyze the amino acid sequences of the known P-type ATPases and predict the membrane-embedded portion of the enzyme as well as the extracytoplasmic loops. Alignment of the different pumps allows definition of minimal length of the connecting loops and at least the extracytoplasmic termination of the membrane-spanning segments. From such alignment for the H^+,K^+ ATPase, the beginning and end of the membrane-spanning segments can be predicted. These data can then be used in a molecular-modeling approach, using the helical arrangement of bacterial rhodopsin as a guide to the directionality of the extracytoplasmic loops (KB Munson, in preparation). A hypothetical arrangement of the first six membrane-spanning segments and their intervening loops is shown in Figure 4*A*. Highlighted in this figure is the M5-loop-M6 sector which contains several hydrophilic amino acids including 3 carboxylic amino acids. This working model allows us to attempt to define the ion binding domains with the membrane sector and to visualize the ion pathway. This is again of interest when the mechanism or design of pump inhibitors is considered.

THE β SUBUNIT The β subunit appears to cross the membrane only once; most of the protein is extracytoplasmic and glycosylated at all of the glycosylation-consensus sequences. In addition to six or seven glycosylated sites, the ex-

tracytoplasmic face has three disulfide bridges. Although reduction of these disulfides inhibits ATPase activity (28), the β subunit probably plays a structural or membrane-targeting rather than a direct functional role. Variation of the β subunit in the Na^+,K^+ ATPase appears to modify K^+ affinity, perhaps altering structure on the extracytoplasmic face of the enzyme (29).

The β subunit is strongly associated with the α subunit, in particular with the M7-loop-M8 sector of the α subunit (30). In the case of the Na^+,K^+ ATPase, the C-terminal hydrophobic amino acids of the β subunit are important for the assembly of the β subunit with the α subunit (31). An antibody, mAb 146-111, has been shown to recognize regions of both the α (positions 873–877, just after M7) and the β subunit (probably between positions 161 and 178), which implies association between the two subunits in this region (32).

Biosynthesis and Turnover of the H^+,K^+ ATPase

This is an important topic because currently available drugs that are targeted to the acid pump bind covalently. This may imply that the pump has to be synthesized de novo to reestablish acid secretion, though some loss of compound may also occur. Studies in rats, using protein inhibition with cycloheximide, implied a pump half-life of about 72 h (33). This result was extended by direct measurement of pump turnover, using pulse-chase labeling with ^{35}S methionine (34), giving a half-life of ca 50 h. In the rat, the parietal cell is in a generally stimulated state; most of the pump population is present in the secretory canaliculus. Administration of the H2 receptor antagonist ranitidine prolonged the half-life of the pump to about 125 h (K Gedda, D Scott, M Besancon & G Sachs, unpublished data). The level of ranitidine given would

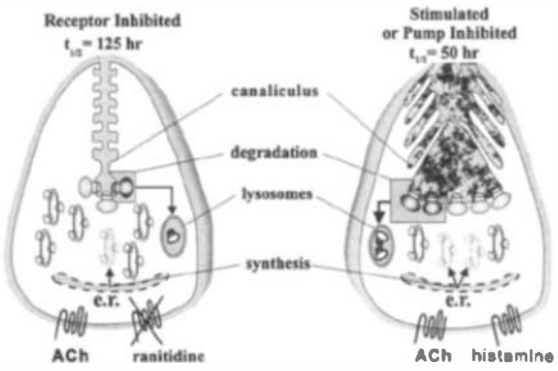

Figure 5 A model of the determinants of the catabolism of the gastric H^+,K^+ ATPase, where we hypothesize that endocytosis and destruction of the pump occur as a function of its presence in the secretory canaliculus and not in the cytoplasmic tubules. (*Right*) The turnover determined for control rats does not differ from that shown for omeprazole treated animals, since the distribution of pump between tubule and canaliculus over a 24-h period is not altered. (*Left*) The prolongation of the half-life after ranitidine infusion occurs because more pump is present in the tubules.

be expected to generate a resting state of the cell; here, the pump is in the cytoplasmic tubules and not associated with the canaliculus. If endocytosis of the canalicular membrane, which forms endosomes and secondary lysosomes, is the major degradative pathway for the pump, then stimulation of acid secretion, which generates more canalicular area, is expected to increase pump turnover, and conversely, inhibition of stimulation of the parietal cell is expected to decrease pump turnover, as occurs with ranitidine. This model of pump turnover is shown in Figure 5. If pump inhibitors change the distribution between tubules and canaliculi, then they should change the half-life of the pump. If they do not change distribution, they should not have an effect on pump turnover.

Membrane Assembly and Targeting of the Gastric H^+,K^+ ATPase

The parietal cell has a basal lateral Na^+,K^+ ATPase and concurrently expresses the gastric H^+,K^+ ATPase either in cytoplasmic tubules or in the secretory canaliculus. The β subunit of the Na^+,K^+ ATPase can act as a surrogate for the α subunit of the gastric H^+,K^+ ATPase and vice versa, in terms of targeting the enzyme to the plasma membrane of the oocyte (36). Further, function of the α subunit of the Na^+,K^+ ATPase may be normal, even when assembled with the β subunit of the gastric H^+,K^+ ATPase. However, in the absence of a β subunit, the synthesized α subunit protein is unstable and does not survive. The β subunit therefore plays a critical role in pump assembly as well as in pump targeting (37).

Some insight into how the β subunit plays this role was derived from studies of secondary structure, either by analysis of mature protein or by analysis of the translation and insertion of different known or putative membrane segments. Whereas analysis of structure of the mature pump provided unequivocal evidence for the existence of 8 membrane segments prior to position 973 and no evidence for a further 2 membrane segments, in vitro translation provided evidence for the first 4 and the last 3 of 10 membrane segments. Hence the membrane segments five, six, and seven did not appear to coinsert during translation. The M8 segment acted as a stop transfer sequence but evidently, in the absence of insertion of M7, was not active in assembly of its neighbour (25).

A hypothesis that might explain these results involves the β subunit in assembly of the α subunit. The β subunit is known to interact strongly with the C-terminal region of M7, and this interaction is perhaps due to interaction with at least one sequence occurring close to cys161–178. If this interaction occurs during translation of the β subunit on the cytoplasmic side of the endoplasmic reticulum (er), then the continuing translation of the β sequence will force membrane insertion of M7, M8 can act as a stop transfer sequence,

and at least 8 of the 10 membrane-spanning segments will have been assembled. If M7 is also able to interact with M5, then M5 and M6 can also insert upon membrane insertion of M7, and the final topology of the α,β heterodimer will be achieved.

The gastric H^+,K^+ ATPase is a pump that is targeted not to the basal lateral but to the apical surface of the parietal cell. When expressed, for example in HK 293 cells, in the absence of a β subunit, the catalytic subunit is retained within the rough er (rer) but is also relatively unstable. In the presence of a β subunit, the α,β heterodimer can move to a smooth membrane fraction that is still retained within a nonpolarized cell. The α subunit is also stabilized by the expression of the β subunit. With polarization, the pump is expressed apically (38). The sorting signals appear to be present both in the C-terminal half of the α subunit and in the β subunit (39). There is no evidence from either the parietal cell itself or the expression systems to indicate that the pump appears in the basal lateral membrane first and then sorts to the apical side. Thus these data argue against the idea that polarized protein distribution is a function of selective retention rather than positive sorting (40). If this were the case, then not only would one be able to find a mixture of the two pumps in the basal lateral surface of the parietal cell, but the pump would also be expressed in the plasma membrane of nonpolarized cells.

Under normal circumstances, the H^+,K^+ ATPase is not exposed on the basal lateral surface of the parietal cell. However, atrophic gastritis and pernicious anemia are associated with circulating antibodies against the H^+,K^+ ATPase (against the α but mostly against the β subunit). Perhaps this results from abnormally high presentation of the enzyme on the basal lateral surface of the progenitor cell, with exposure of the extracytoplasmic domain of the β subunit, or a higher than normal turnover of this cell type generates an autoimmune response.

Catalytic Cycle of the Pump

The gastric ATPase pumps H^+ in exchange for K^+, much in the same way as the Na^+,K^+ ATPase pumps Na^+ in exchange for K^+. These enzymes appear to share many of the steps and conformational changes that accompany transport. By definition, the enzyme conformations in which the ion-binding sites face the cytoplasm are considered E_1 conformations, the conformations in which the ion-binding sites face the outside are considered E_2 conformations, and the conformations in which the ion is trapped within the protein are designated occluded conformations. In simple terms the pump protein cycles between E_1 and E_2 forms, accompanying phosphorylation and dephosphorylation and binding, transport, and release of ions. A more complete catalytic scheme is shown in Figure 6, which shows the different conformers (41).

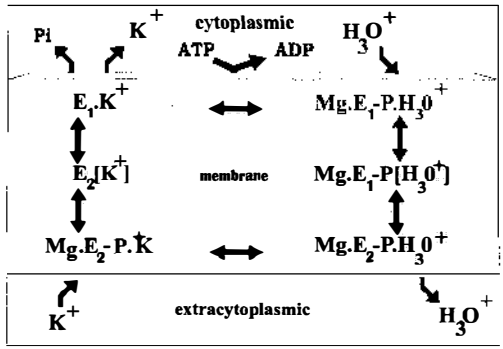

Figure 6 The catalytic cycle of the gastric H^+,K^+ ATPase. In the presence of H^+, the pump is phosphorylated in the presence of MgATP, and hydronium ion H_3O^+ is transported outward as a function of the conversion of the E_1-P to the E_2-P form, via the occluded form E-P[H_3O^+]. The binding of K^+ to the extracytoplasmic face of the pump results in dephosphorylation, loss of bound Mg, and conversion back to the $E_1.K^+$ form, via the occluded E.[K^+] form.

The pump extrudes H_3O^+ at a concentration of 160 mM, and reabsorbs K^+ into a cytosolic concentration of about 140 mM. Again, this transport effect can be compared to that of the Na^+ pump, which does much the same thing with respect to Na^+ and K^+. In contrast to the Na^+ pump that generally extrudes Na^+ into the bathing medium, the gastric acid pump extrudes H^+ into a membrane-enclosed space within the parietal cell—the lumen of the secretory canaliculus. Acid reaches the gastric lumen through a restricted pore at the apex of the canaliculus. Hence the acidity of the canaliculus is extremely high, reaching a pH of 0.8, which is equivalent to the 160-mM HCl elaborated by the pump.

The rate-limiting step of the pump in the absence of extracytoplasmic K^+ is dephosphorylation. However, with K^+ present in the lumen of the canaliculus, the rate-limiting step is probably the transition from the phosphorylated E_1 form with occluded proton (hydronium) to the E_2 phosphorylated form with proton bound (right-hand side of Figure 6).

The pump is present in the membrane of the cytoplasmic tubules and in the secretory canaliculus. In the former location it does not transport protons at a significant rate. In the latter the pump is fully active. The transition between these two states is determined by access of K^+ to the luminal face of the pump. It has been shown that activation of HCl secretion is accompanied by activation of a K^+ plus Cl^- conductance in the canalicular membrane (9). It is still unclear whether this KCl pathway remains a property of the canalicular membrane, and the pump separates from the pathway in returning to the tubular state or whether the pathway is present in the cytoplasmic tubules and activates along

with the morphological transition and inactivates with pump retrieval. However, major cytoskeletal elements are involved in the transition between resting and stimulated states of the parietal cell (42).

Organ Distribution of the Gastric H^+,K^+ ATPase

The colonic ATPase is apparently an H^+ for K^+ exchange enzyme, but it differs structurally from the gastric H^+,K^+ ATPase in that there is only 75% sequence homology (14). On the other hand, several lines of evidence, including direct cloning of the α and β subunits from rat kidney, indicate the presence of a protein idential to the gastric pump, probably in the collecting duct (43, 44). Transgenic tagging of the β subunit has also shown that this subunit is expressed in the renal collecting duct (45). The function of the gastric or colonic H^+,K^+ ATPase either in kidney or colon, respectively, is unlikely to be one of acidification of the lumen or excretion of metabolic acid, since these organs are also endowed with V-type ATPases that can be present in the apical membrane. The H^+,K^+ ATPase in these structures is more likely responsible for K^+ conservation rather than with proton extrusion.

PUMP INHIBITION

General Considerations

The structure of the catalytic subunit of the pump and its transport function suggests that inhibitors of the enzyme should be designed to react either extracytoplasmically or within the membrane domain of the enzyme. Given that the gastric H^+,K^+ ATPase is a member of a large family of ion-transporting ATPases, many of which carry out essential cellular functions, the inhibition must be pump-type specific in order to be clinically useful.

Certain physical and chemical properties of inhibitors can take advantage of the highly acid space generated by the acid-transporting pump. Thus a protonatable weak base will be concentrated in the acid space of pH 1.0 in the canaliculus. However, there are other acid spaces in the body with a pH of about 5.0, and it is wise to target only the canalicular space. This means that the drug should have a pK_a of 5.0 or less, since lysosomes, neurosecretory granules, and endosomes have an internal pH of about 5.0. This consideration will also avoid the targeting of the H^+,K^+ ATPase present in the kidney, in that there will be preferential concentration of the drug in the parietal cell, given the lower pH of the canalicular space.

The extracytoplasmic domain of the catalytic subunit of the H^+,K^+ ATPase is relatively small and must contain the binding site for K^+, so many of the reversible type of inhibitors may turn out to be K^+ competitive.

The acidic nature of the space suggests that it might be possible to design

acid-activated compounds that become reactive only after acid activation. In terms of drugs that are available or currently in development, all take advantage of the acidic space. Some are acid-activated prodrugs; others are K^+ competitive. All are protonatable weak bases.

The Pyridyl Methylsulfinyl Benzimidazoles

The pyridyl methylsulfinyl benzimidazoles are covalent inhibitors of the gastric H^+,K^+ ATPase. The structure of the different compounds approved for use in humans, as well as the mechanism of activation of these compounds is illustrated in Figure 7 (46–49).

These compounds are protonatable weak bases; the pyridine N has a pK_a of about 4.0. The benzimidazole N pK_a in these compounds is probably about 2.0. Thus these compounds will concentrate in acidic spaces in the body, provided that the pH within these spaces is less than 4. The only known acidic space with this low a pH is the secretory canaliculus of the active parietal cell, which has a pH of 1.0 or less. Theoretically, therefore, these compounds will accumulate more than one thousandfold in this space, prior to their inhibition of the proton pump. Substitution on the pyridine ring is designed so as to move the pK_a of the pyridine N to ~4.0. Substitution on the benzimidazole ring may have effects on the pK_a of the benzimidazole N, which may in turn determine the neutral pH stability of the compounds.

Following accumulation in an acidic space, these compounds undergo an acid-catalyzed conversion to a cationic, tetracyclic sulfenamide, as illustrated in Figure 7. This reactive species is able to bind to accessible cysteines on the H^+,K^+ ATPase.

The formation of the tetracyclic species from the protonated form of the substituted benzimidazoles is the rate-limiting step in the formation of the active species from the prodrug. Different hypotheses can be considered, both to explain the acid activation of the reaction and also to explain reactivity at neutral pH. The C2 on the benzimidazole reacts only with the unprotonated N of the pyridine ring, which is the main reaction at neutral pH. One hypothesis for acid activation is that protonation of the benzimidazole N in acidic solutions increases the reactivity of the C2, facilitating attack by the unprotonated pyridine N. This N has to deprotonate at relatively acidic pH values in order to account for the transformation to the sulfenamide. To facilitate this deprotonation, an intramolecular transfer of the proton from the pyridine N to the benzimidazole N may occur, as shown in Figure 7. This transfer may be due to H bonding with the sulfoxide moiety. Nuclear magnetic resonance (NMR) spectral data of omeprazole as a function of pH has directly shown that the pyridine N is the first site of protonation. The 3-Me group on the pyridine shows a large high-field shift as a function of decrease of pH, suggesting that this group moves into the vicinity of the benzimidazole and accelerates reaction

Figure 7 The chemistry of the pyridyl methylsulfinyl benzimidazoles: lansoprazole, omeprazole, and pantoprazole. This figure illustrates accumulation as a function of protonation, conversion to the tetracyclic sulfenamide, and reaction with accessible cysteines on the H^+,K^+ ATPase.

with the C2 of the benzimidazole (49a). At extremely low pH, where both the pyridine and the benzimidazole are protonated, the reaction is once again slowed, presumably because the deprotonation of the pyridinium is slower. Therefore, although the tetracyclic sulfenamide has been established as the active intermediate in inhibition of the ATPase, the pathway involved in generation of this intermediate is not well defined.

Substituents on the benzimidazole ring have resulted in differences in stability at neutral pH. For example, the difluoromethoxy group present in pantoprazole slows reaction at neutral pH by electron withdrawal, which decreases the pK_a of the benzimidazole N and reduces its protonation at all but highly acidic pH values. In terms of stability at neutral pH, pantoprazole is therefore more stable than omeprazole, which in turn is more stable than lansoprazole, which in turn is more stable at neutral pH than a compound currently under investigation, E3810 [2-(4 methoxypropyloxy 3 methyl) pyridyl methylsulfinyl bezimidazole]. At acidic pH (pH = 1), the rate of sulfenamide formation for all four compounds is very similar.

The sulfenamide is very unstable at neutral pH and relatively stable at acidic pH. Further, the sulfenamide is a permanent cation, reducing its membrane permeability and therefore its entry into the parietal cell. Because of neutral pH instability and membrane impermeability, the sulfenamide is targeted to the extracytoplasmic domain of the H^+,K^+ ATPase.

In the presence of thiols, a diffusion limited reaction occurs between the sulfenamide and the thiol. Thus, if the sulfenamide is formed in bulk solution, the only expected limitation of reaction with cysteines of the pump is their accessibility. Binding of the drugs to cysteines of the α subunit of the enzyme has been directly demonstrated by labeling studies followed by sequencing. The label is displaced entirely by reducing agents, and full reversal of enzyme activity and acid transport is achieved by using low concentrations of the relatively hydrophobic SH reagent tributylphosphine (M Besancon & G Sachs, unpublished observations). Inhibition by any one of the pyridyl methylsulfinyl benzimidazoles results in an increased proton leak across the enzyme, which is also reversed by treatment with tributylphosphine. Less hydrophobic reagents are only partially effective, presumably because there is a reduction of the disulfides of the β subunit along with removal of the reacted drug (M Besancon & G Sachs, unpublished observations). The site of reaction important for inhibition or generation of leak across the enzyme must therefore be relatively hydrophobic.

There is unexpected selectivity in terms of the cysteines that react with the different compounds. Lansoprazole reacts with three cysteines: cys321, -813 (or -822), and -892 (50); omeprazole reacts with two cysteines: cys813 (or -822) and -892 (24); and pantoprazole reacts with both cys813 and -822 (51). This unexpected pattern must be due to a selectivity of reaction or access that

is unexpected in the sulfenamide chemistry. It has not been possible as yet to separate cys813 or -822, but one of these is labeled by lansoprazole and omeprazole, and both are labeled by pantoprazole. From modeling studies, cys813 seems to be more accessible than cys822, which makes it the likely residue derivatized by lansoprazole and omeprazole. We therefore assume in the subsequent sections that cys813 is the amino acid reacting with these drugs.

Recent experiments have shown that labeling cys813 but not cys892 by omeprazole is essential for inhibition by this drug (M Besancon, unpublished observations). Because labeling of cys813 is common to all three drugs, disulfide formation with this cysteine may be important for inhibition by the substituted benzimidazoles. Figure 4*B* illustrates the binding of omeprazole at cys813. This postulate is also consistent with the need for a relatively hydrophobic reagent for full reversal of inhibition and transport activity.

Cysteine 892 is predicted to be in the long, hydrophilic extracytoplasmic loop between M7 and M8. This cysteine is likely to be relatively more exposed than cys813, which would account for its more rapid reaction with omeprazole. Cys813 is modeled as being buried within the extracytoplasmic domain, relatively shielded from the lumen of acidic space. Cys321 is predicted to be at the extracytoplasmic surface of M3, and cys822 within M6. Most surprising is the absence of reaction of pantoprazole with cys892 and its unique ability within these three drugs to react with cys822. In fact, only pantoprazole binding changes the tryptic accessibility of the cytoplasmic side of M5, where the cleavage site moves from position 776 to 792 as a function of pantoprazole binding (51). Presumably, binding to the second cysteine in M5-loop-M6 determines this conformational change induced by reaction with the pantoprazole sulfenamide. Pantoprazole is the most stable of the three drugs studied in terms of binding to extracytoplasmic cysteines in acid-transporting vesicles. One possibility for the peculiar selectivity of pantoprazole is that binding of the protonated species occurs prior to the formation of the sulfenamide. Sulfenamide formation then occurs within the extracytoplasmic domain of the enzyme in the proton pathway, perhaps catalyzed by a group within the binding site of the protonated prodrug. Steric factors may also be invoked as an explanation. If this is the case, it is not only the chemistry of the pyridyl methylsulfinyl benzimidazoles that determines reaction, but reaction is also determined by the structure of the extracytoplasmic domain of the pump.

The rate of inhibition in vesicles that achieve a pH of ~1.0 following the addition of ATP is also surprisingly slow. One would anticipate a rapid conversion to the sulfenamide, within 1–2 min. If the sulfenamide in free solution were able to react with the cysteine(s) essential for inhibition, one might expect inhibition to be complete within 1–2 min, given the above rate of formation of the sulfenamide at pH 1.0. However, the $t_{1/2}$ of inhibition of acid transport in isolated gastric vesicles at 20-μM compounds is ~300 s for lansoprazole,

~450 s for omeprazole, ~700 s for pantoprazole, and somewhat longer for inhibition of ATPase activity. Some of the ATPase is present in vesicles that achieve a lower internal pH, and binding of the drugs to the pump induces a proton leak across the pump—a leak that is reversed along with restoration of ATPase activity and acid transport by the hydrophobic SH-reducing agent, tributyl phosphine.

Different explanations for these selectivities are possible. First, once the sulfenamides are formed, the time of onset of inhibition could reflect slow access to the critical cysteine, owing to steric hindrance. Cys892 is predicted to be in a hydrophilic region of the extracytoplasmic domain and therefore would be expected to react more quickly. In fact, reaction with labeled omeprazole has shown that cys892 does react much faster than cys813, and reaction with the latter correlates with inhibition. If this were the only explanation, the lack of reaction of cys892 with pantoprazole would be difficult to explain. Therefore it may be that the protonated species binds to the pump and forms a sulfenamide within the structure of the extracytoplasmic domain. The sulfenamide derived from pantoprazole is then able to react first with cys822 and then with cys813; a reaction with either probably results in inhibition.

Pharmacokinetics of Secretory Inhibition by the Substituted Benzimidazoles

It has been shown that omeprazole reacts only with the pump present in the secretory canaliculus (10). This is (*a*) because acidification is necessary for both concentration of the drug and activation to the sulfenamide and (*b*) because only the pumps in the canalicular membrane are forming HCl. The half-life of the substituted benzimidazoles in blood is between one and two hours; hence exposure of pumps to the drug is limited. Following a meal, not all of the pumps are active, and only the pumps that are active during the effective plasma levels of the drugs will be inhibited.

The half-life of the pump in rat (and of acid-secreting inhibition in humans) is 50 h (34). Thus, on once-a-day dosing, significant amounts of newly synthesized pump that have not been exposed to omeprazole will be present 24 h after dose. From the half-life, about 25% of the pumps are synthesised over a 24-h period. This is probably the major mechanism for recovery of acid secretion following covalent inhibition of the pump by the substituted benzimidazoles. Perhaps there is some recovery due to reduction of the disulfide bond formed following sulfenamide formation. However, this requires either glutathione secretion into the canaliculus or exposure of the extracytoplasmic domain during pump cycling between cytoplasmic and canalicular sites. The finding that cycloheximide abolished restoration of acid secretion in the rat following omeprazole treatment argues that this latter pathway is of minor importance (33).

Assuming that maximally only 75% of the pumps, old or new, are active during the presentation of the compounds, steady-state acid secretory capacity (ca 30%), as reflected by the number of active pumps, should be achieved at about 48 h following initiation of therapy. However, very significant inhibition will be found even after the first dose, which is more than that achieved with H2 receptor antagonists.

It is difficult to predict the change of intragastric pH dependent on the reduction of the number of active pumps. In humans, the mean diurnal pH is about 1.4 to 2.0; low pH is mainly found at night. The target pH for optimal healing of duodenal ulcer disease is a pH greater than 3.0 for 16–18 h, i.e. about 75% of the time. This is achieved for 20% of the time in untreated controls, and is achieved after 2 or 3 days treatment with omeprazole, and probably with the other substituted benzimidazoles. Given that the acid secretory capacity falls to about 30% based on the number of residual active pumps with a single daily treatment, this target pH and duration may occur even on day 2 of once-a-day therapy, as illustrated in Figure 8. For circumstances in which better pH control is warranted, twice-a-day treatment may achieve the desired pH somewhat later.

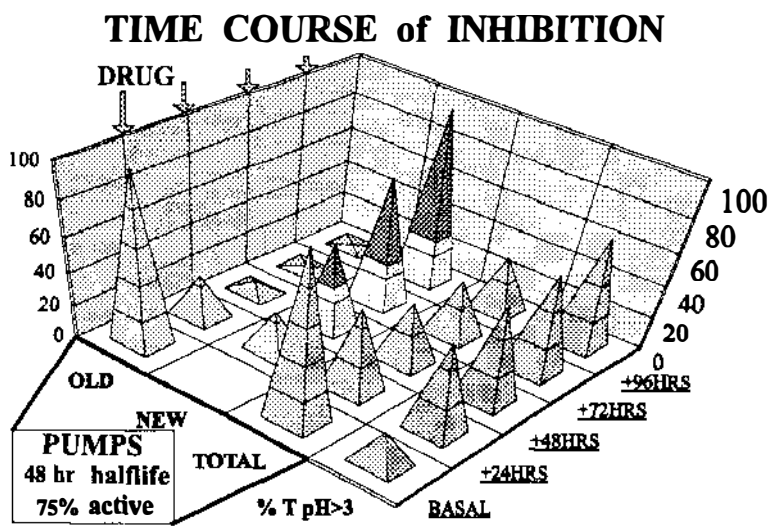

Figure 8 The predicted effect of once-a-day dosing with the pyridyl methylsulfinyl benzimidazoles. The assumptions made are that the half-life of the pump is 50 h, that de novo synthesis is required for restoration of acid secretion, and that at time of dosage only ~75% of the pumps are active.The first row of pyramids shows the number of original pumps that are active; the second row, the pumps synthesised in the absence of drug in the plasma, with shading for those new pumps inhibited by drug; the third row, the total number of pumps available; and the fourth row, the predicted percentage of the day at which intragastric pH is elevated beyond 3.0.

To improve acid control, given the above ideas, it is more appropriate to increase dose frequency rather than quantity. Once maximal inhibition of active pumps has been achieved, newly synthesized or newly active pumps will not be inhibited three hours after dose, since the drug is no longer present in blood, and a second dose will be required along with meal stimulation to inhibit these pumps. On twice-a-day dosing with meals, inhibition should be more rapid and greater, so maximal secretory capacity will be reduced to about 10%, and pH elevation will also be greater, perhaps reaching a luminal pH of about 5 for 16 h. Wall pH can be significantly lower than luminal pH.

K^+ *Competitive Antagonists*

A series of compounds that contain protonatable nitrogen moieties have been shown to inhibit the gastric H^+,K^+ ATPase (53–55) (Figure 9). In particular, a (1,2 α) substituted imidazopyridine, SCH28080, was shown to inhibit acid secretion in animals and in humans (56). Investigation of its mechanism showed that this compound was K^+ competitive and bound with higher affinity in the protonated form with the extracytoplasmic surface of the enzyme (57, 58). Several other compounds based on this structure have been studied with improved bioavailability as compared with SCH 28080 and a reduced toxicological profile (59).

The aryl quinolines (60), which are a series of compounds of quite different structure, have also been shown to be K^+-competitive reagents selective for the gastric H^+,K^+ ATPase, as compared with the Na^+,K^+ ATPase. These compounds also contain protonatable nitrogens and react with the extracytoplasmic face of the enzyme. One of these, MDPQ, is fluorescent and changes its fluorescence with respect to both quantum yield and E_{max}, when bound to the enzyme, as if its environment was more hydrophobic when bound. Phosphorylation of the enzyme further increases its fluorescence, and the fluorescence is quenched by extracytoplasmic K^+ and by SCH 28080 (61). Apparently this arylquinoline reports on a conformational change occurring in its binding domain.

The region of binding of the imidazopyridines has been elucidated by using an azido- and N-methylated derivative of SCH28080, Me-DAZIP$^+$ (62). Photoaffinity labeling with this compound showed that it was saturable and K^+ competitive. Following labeling and trypsinolysis, it was shown to be bound to the region of the enzyme containing the M1-loop-M2 sector (62). This region corresponds to the region important for binding ouabain in the homologous Na^+,K^+ ATPase (63). The extracytoplasmic loop is rich in carboxylic acids, and the protonated N of the K^+ competitive antagonists may interact with these carboxylic acids.

Detailed structure-activity relationships have been worked out for the imidazopyridine series (64). The molecule apparently requires an imidazopyridine (with limited substitution on the two and three positions), an aliphatic link,

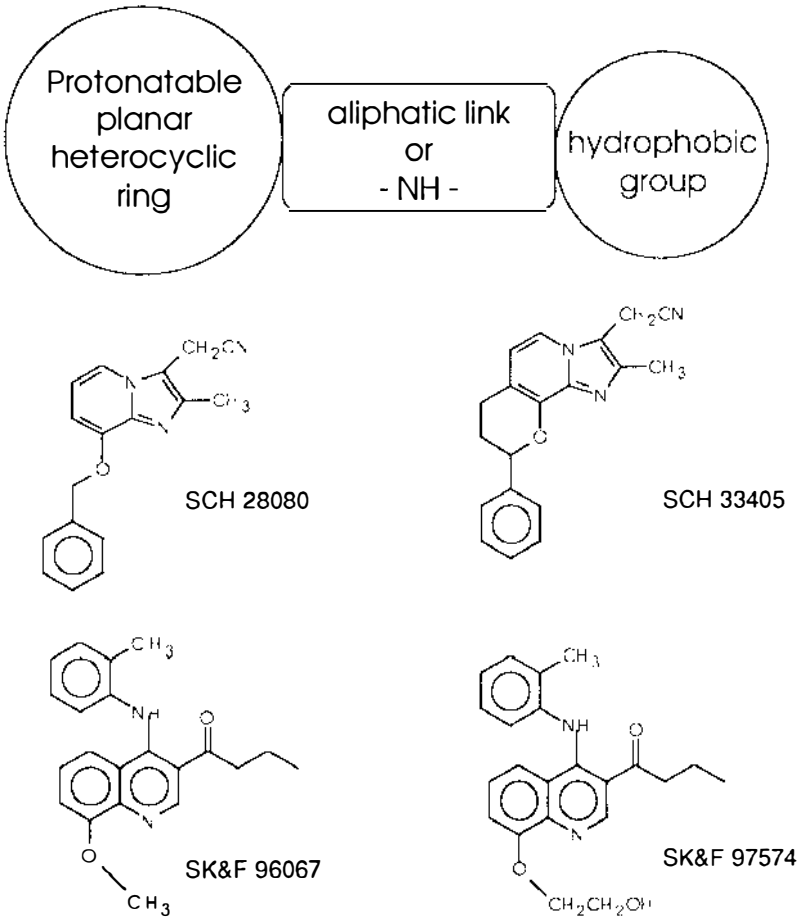

Figure 9 The structures of two substituted imidazo (1,2 α) pyridines, SCH 28080 and SCH 33405 (a constrained analog) and two arylquinolines that have been given to man. Above is the proposed general structure for high affinity K^+ competitive inhibitors.

and a hydrophobic ring orthogonal to the imidazopyridine. A search of the structure of the M1-loop-M2 region led to the suggestion that the docking sites were asp 136 and phe 124, but many other possibilities can be envisaged.

SCH28080 binds to both the phosphorylated form (with high affinity) and to the dephosphorylated form of the enzyme (65, 66). The stoichiometry of binding appears to be between one and two moles compound per mole phosphoenzyme. The azido derivative, Me-DAZIP$^+$ appears to bind with a stoichiometry close to one (62). Both the imidazopyridines and the arylquinolines

are highly selective for the H^+,K^+ ATPase, as compared with the Na^+,K^+ ATPase.

Several of these K^+ competitive compounds are under development. Two aryl quinolines, SK 96067 and 97574, have been given to humans, and the former results in a dose-responsive inhibition of acid secretion that is greater than that achieved by H2 receptor antagonists and, in a subset of those treated, equivalent to that of the substituted benzimidazole omeprazole (67). Other imidazopyridines and arylquinolines are also under consideration for clinical trials.

Covalent as well as reversible pump inhibitors are dependent on an acidic space for concentration at their site of action. The pyridyl methylsulfinyl benzimidazoles are converted to the active sulfenamides, which react covalently with the active fraction of the H^+,K^+ ATPase. They have a short plasma half-life limiting their effect on inactive pumps with the first dose, and a second or third dose is required to achieve steady-state inhibition. Steady-state inhibition should be achieved with the first dose of K^+-competitive drugs, since these compounds do not depend on activation to a covalent inhibitor by an acid space; hence they should inhibit all pumps as long as they are present in plasma. On the other hand, since their binding is noncovalent, their duration of action will depend entirely on their plasma half-life. It will be interesting to discover which acid-related disease is treated best by the covalent type of proton-pump inhibitor and which is treated best by the reversible acid-pump antagonists. Both types of compound, however, should show superiority of acid control compared to receptor antagonists.

CLINICAL CONSEQUENCES OF PUMP INHIBITION

Many patients have been treated with the pump inhibitors omeprazole (85 million), lansoprazole (4 million), and more recently, pantoprazole. Generally, these compounds, as compared with H2 receptor antagonists, produce a more profound inhibition of acid secretion on once-a-day therapy.

Various comparative studies all agree that pump inhibitors produce superior healing and symptom relief in esophagitis (68–70). Most studies of pump inhibitors also show superior healing and pain relief in duodenal ulcer disease compared with H2 receptor antagonists (71–73). In the case of gastric ulcers, the data are also in favor of pump inhibitors; a majority of comparative studies have also shown superiority for this category of drug (74).

Since acid-induced damage to either the esophagus or the duodenum depends on luminal acid, pH metry of the stomach contents allows reasonable prediction as to the probable efficacy of antiulcer therapy (1, 2). However, acid-induced damage to the gastric mucosa probably depends more on acidification within the epithelium; hence intragastric pH metry is a less useful

parameter. If it is indeed intramural pH that determines healing of gastric ulcers, a more complete inhibition of the acid pump is likely to be required than for either esophagitis or duodenal ulcer disease. To achieve this level of inhibition, twice-a-day dosing with the covalent pump inhibitors is probably necessary.

In contrast to H2 receptor antagonists, inhibition of acid secretion is independent of the pathway of stimulation. Tolerance to the substituted benzimidazoles does not occur, and acid does not appear to rebound after short-term treatment.

SIDE EFFECTS OR ADVERSE EVENTS DUE TO PUMP INHIBITORS

The presence of acid in the antrum activates somatostatin release from the antral D cell, which in turn inhibits gastrin release from the G cell. The G cell is stimulated to release gastrin by the presence of amino acids in the antrum. If acid secretion is inhibited in the presence of food, the loss of the acid brake on gastrin release will result in hypergastrinemia. Life-long high levels of gastrin in the rat results in ECL cell hyperplasia and eventually in the formation of nests of ECL cells called carcinoids (75). The formation of carcinoids has been of concern in the use of pump inhibitors for human disease. It appears that there is no direct drug action on ECL cells (76) but that all the effects can be accounted for by hypergastrinemia (77). Nevertheless, several studies that monitored patients for up to nine years were carried out in humans who had been receiving high doses of omeprazole (78). It seems that even ECL cell hyperplasia was not really found in these patients; the small increase in ECL cell volume occupancy was due to progressive gastritis that appears to accompany the disease (78).

This effect on ECL cells is probably the only real side effect of treatment with these compounds that are of possible significance in humans. Many other attempts have been made to show the presence of side effects or adverse events related to the substituted benzimidazoles. They have all been shown to be without merit. For example, it was claimed that evidence had been found for the genotoxicity of omeprazole or its products. These experiments were based on a false assumption—that pronase digestion of gastric mucosa does not isolate progenitor cells—and have since been withdrawn by the authors (79), as well as refuted by other laboratories (80). Induction of cytochrome P450 1A1 and 1A2 has been shown, and claimed to be a danger to humans. However, these same cytochromes protect against, rather than induce, cancer (81). More recently it has been suggested that omeprazole has adverse effects on vision. Again, there is a plethora of data that show that omeprazole does not affect

vision in animals or humans. Given the 85 million cases treated with omeprazole, it seems highly unlikely that any significant problems will arise with the further use of this drug. Another antiulcer drug, ranitidine, did result in retinopathy in four dogs and has a half-life in the retina of about six months. The other proton-pump inhibitors approved for clinical use have the same spectrum of action as omeprazole, but the safety experience, although expanding, is still limited.

THE SUBSTITUTED BENZIMIDAZOLES AND *HELICOBACTER PYLORI*

A substantial body of evidence now exists showing that duodenal or non–NSAID-induced (NSAID, nonsteroidal antiinflammatory drugs) gastric ulcers require the presence of both acid and infection with *Helicobacter pylori* (82, 83). How *H. pylori* contributes to the development of ulcer disease is unknown. A likely explanation is the loss of tight junction integrity induced by the presence of the organism. Many arguments favor this hypothesis. H^+ back diffusion across the apical membrane of acid-exposed tissue is likely to be small. Proteins capable of proton transport are likely to be absent from this surface, and the inherent proton permeability of the lipid bilayer, although about one order of magnitude greater than that for Na^+ or K^+, is still such that any back diffusion can be balanced by proton-export mechanisms across the basal lateral surface of the cell. The tight junction is normally relatively permeant to ions as compared with the apical membrane of the cell. Peptic cell monolayers are highly resistant to acid on their apical surface, for example (84). Tight junction integrity is maintained by a protein, the tight junction protein (TJP). The number and complexity of TJP strands and the defects in association between neighboring TJP strands probably determine the ion permeability of the tight junction region. If *H. pylori* were able to alter the characteristics of tight junctions in certain individuals, ulcers would result if acid were present on the luminal surface. Since it appears that the esophageal epithelium does not resist acidity, *H. pylori* does not contribute to esophagitis. Hence a combination of the presence of acid and the presence of *H. pylori* would be required for gastric and duodenal ulcer.

It is now believed that primary treatment of duodenal and gastric ulcer patients should involve not only acid control to allow the lesion to heal but also eradication of *H. pylori* to prevent recurrence (85). The issue is how to eradicate this organism.

The average ambient pH in the normal human stomach over a 24-h period is 1.4. However, the pH at the surface of the gastric mucosa may be consid-

erably higher, perhaps as high as 5 or 6. This high pH may be due to a mucous barrier, preventing back diffusion of protons; a phospholipid surfactant barrier, also preventing acid back diffusion; or HCO_3^- transport into the mucosal solution, thereby neutralizing the back diffusing acid (86). It is extremely unlikely that even a combination of all three processes could completely counteract proton back diffusion from a solution whose average proton concentration exceeds 50 mM.

Therefore *H. pylori* probably survives at acidic pH values, although it can also survive and grow at neutral pH. It should therefore be thought of as a facultative acidophile. *H. pylori* occupies an unique niche in mammalian biology, the acidic environment of the stomach. Eradication therefore requires access of an antibiotic into the organism and into the stomach.

Two therapies have been much discussed, triple or quadruple therapy (bismuth, metronidazole, and amoxycillin ± ranitidine) (87) and dual therapy, which involves a substituted benzimidazole and either amoxycillin or clarithromycin (88). Metronidazole resistance occurs in about 20% of patients, and triple or quadruple therapy requires taking 11 tablets a day for 2 weeks followed by ranitidine for another 4 to 6 weeks. Clearly patient compliance will be a problem with this approach.

Dual therapy (namely a proton-pump inhibitor with a single antibiotic) is less daunting; it requires, depending on the exact regimen, only up to five tablets per day (88). Nevertheless, compliance may still be an issue. Further, it is unknown why omeprazole, for example, improves the response to either amoxycillin or clarithromycin. At first researchers believed that the drug had a direct action on *H. pylori*, since it was possible to show in vitro that the substituted benzimidazoles were able to kill *H. pylori* when growing at neutral pH. As it turns out, this effect is not due to the drug itself but rather reflects the effect of breakdown products, which in turn reflect instability of the chemicals at neutral pH. The effect of the benzimidazoles is more likely due to the elevation of gastric pH. This elevation of pH now makes the organism sensitive to antibiotics, to which it is insensitive at the normal acidic pH of its environment. Whether there is better access of antibiotic to the interior of the organism at neutral pH or whether metabolic or synthetic pathways alter in the organism to render it sensitive at neutral pH is still unknown. Both amoxycillin and clarithromycin require growth of bacteria to exert their lethal effect, and perhaps, the higher pH achieved with proton-pump inhibition allows the growth necessary for eradication.

Although proton-pump inhibitors will probably be used in combination with a single antibiotic to eradicate *H. pylori* in the ulcer patient, the major breakthrough in eradication will come when a single drug is developed that is capable of eradicating the causative factor for both peptic ulcer disease and the development of gastritis.

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