The Novel Acid Pump Antagonists for Anti-secretory Actions with Their Peculiar Applications Beyond Acid Suppression

Marie Yeo,¹ Dong Kyu Kim,¹ In Sik Chung,² Byoung Seok Moon,³ Keun Seog Song,³ and Ki-Baik Hahm¹*¹

¹Genome Research Center for Gastroenterology and Department of Gastroenterology, Ajou University School of Medicine, Suwon 443-721, Korea
²Kangnam St. Mary Hospital, Catholic University School of Medicine, Seoul 137-701, Korea
³Yuhan Corp., Seoul 156-754, Korea

Received 28 October, 2005; Accepted 31 October, 2005

Summary  The H⁺/K⁺-ATPase of gastric parietal cell exchanges luminal K⁺ for cytoplasmic H⁺, of which outcome is gastric acidification with outflux of hydronium ion (H₃O⁺). Secretion of gastric acid from the H⁺/K⁺-ATPase is stimulated by neuronal sensing and elaborately regulated various neuronal transmitters and hormones, consequently resulting in anchoring of the H⁺/K⁺-ATPase in canaliculi membrane of gastric parietal cell. Since hypersecretion of gastric acid or a defect of its barrier function is considered as a principal casual factor in the acid-related diseases such as duodenal and gastric ulcer, reflux esophagitis, and some types of gastritis, the development of anti-secretory agents including PPIs (proton pump inhibitors) and H₂-RAs (histamine type 2 receptor antagonists) has revolutionized during the second millennium. Similar considerations applying to design of compounds substituting K⁺ led to the development of acid pump antagonists (APAs), of which advantages are independent of secretory status, no lag time required, reversible in actions allowing “on-demand dosage”. Our recent studies revealed that these inhibitors of H⁺/K⁺-ATPase could be extensively applied for the selective induction of cancer cell apoptosis, a significant anti-inflammatory and gastro-protective action beyond acid suppression. In the current review, we described the mechanistic regulation of gastric acid secretion with pump inhibitor, the difference and characteristics between PPI and APA based on the molecular mechanism, and their new applications beyond acid suppression.

Key Words: Gastric acid secretion, Acid pump, Helicobacter pylori, PPI, APA

Introduction

Gastric acids are necessary for sterilization and digestion of food, and specially required for maintenance of the activity of pepsin through the activation of pepsinogen.

They are fundamentally produced by specialized gastric proton pump, H⁺/K⁺-ATPase, presented in parietal cells of gastric glands. The enzyme exports one H⁺ ion and imports one K⁺ ion with consuming one ATP molecule, by which H⁺/K⁺-ATPase is commonly annotated than simple nomination of proton pump [1, 2]. In the resting status, since H⁺/K⁺-ATPases exist in the tubulovesicles of parietal cells of which membrane is impermeable to K⁺ ions, H⁺/K⁺-ATPase stays as inactive form in the vesicles. Hormones such as histamine, acetycholine, and gastrin

*To whom correspondence should be addressed.
Tel: +82-31-219-4383  Fax: +82-31-219-4399
E-mail: hahmkb@hotmail.com
omeprazole was followed thereafter. The sulfide added to the pyridine-2-thoacetaminde of an antiviral agent prodrugs and covalently bind cysteine residues of the gastric PPIs have a unique mode of action, being acid-activated by histamin-2-receptor antagonists (H₂/KA-, K⁺-ATPase inhibitors) [9–11]. As results, a benzimidazole ring was added to the pyridine-2-thoacetaminde of an antiviral agent proved to possess a strong antisecretory activity. The compound accumulated in the acid space the secretory canaliculus of the stimulated parietal cell and consequently converted to the active form, a cationic thiophilic reagent. These activated species binds covalently to one or more cysteines accessible from the luminal face of the pump, interfering outflux of hydronium ion from cytoplasm. Similar considerations applying to design of compounds substituting K⁺ on the outside surface led to the development of acid pump inhibitors (PPI) [12–14]. Whereas the PPIs have a unique targeting and covalent inhibitory action on the proton pump based on their chemistry and the biology of the parietal cell, another class of compound, APA, have a structural specificity for their target region on the pump, close to the K⁺ binding region of the H⁺/K⁺-ATPase and are, thus, K⁺ competitive and dissociate from the pump when their blood concentration drops. Therefore, this agent have great advantages in terms of independent of secretory status, no lag time required, reversible in actions, and could be therapeutic antacids, allowing “on-demand dosage”.

Recently, we have found that the H⁺/K⁺-ATPase inhibitors including PPI and APA could regulate intracellular signaling pathways of apoptosis as well as inflammatory propagation [15, 16]. The studies also revealed that APA seems to be superior against Helicobacter pylori infection, based on the findings that they exerted significant anti-inflammatory, gastroprotective with overt molecular actions. Taken together, our novel findings shed lights on that gastric proton pump inhibitors including APAs could be extensively used for a wide spectrum of gastric diseases comprising of therapeutic approach for gastric inflammatory disease as well as cancer.

The Gastric Acid Secretion and Its Suppression by the H⁺/K⁺-ATPase Inhibitors

The regulation of gastric acid secretion

The M. Yeo et al.

promote the fusion of tubulovesicles with apical membrane of parietal cells, which results in elongation of microvilli and up to 10-fold expansion of the apical membrane area. H⁺/K⁺-ATPase in the fused tubulovesicles are thus exposed to luminal fluid containing K⁺ ions and subsequently the enzyme is activated [3, 4]. Mis-regulation of acid secretion can cause several acid-related diseases of gastric, esophageal, and duodenal tissues, resulting in gastroduodenal ulcer, erosive reflux gastritis, and Zollinger-Ellison syndrome in extreme case. Besides of these hypersecretory conditions, the acid related diseases of the upper gastrointestinal tract can be also caused by a defect in barrier function of the esophageal epithelium in the case of GERD or of the gastric mucosal or duodenal epithelium in the case of gastric or duodenal ulcers [5, 6].

Treatment for acid-related disease has been revolutionized by histamin-2-receptor antagonists (H₂:RA) that significantly suppress gastric-acid secretion and they have been used extensively for more than 25 years [7, 8]. The concept that inhibition of the pump itself would be a more effective way of controlling acid secretion rather than the blockade of one of stimulatory signals led a development of proton pump inhibitor (PPI) [9–11]. As results, a benzimidazole ring was added to the pyridine-2-thoacetaminde of an antiviral agent proved to possess a strong antisecretory activity. The sulfide of the compound was modified to a sulfoxide for stabilization, by which timoprazole, the word’s first proton pump inhibitor, was born and the evolution into the invention of omeprazole was followed thereafter.

As for the exact mechanism of acid inhibition by PPI, the PPIs have a unique mode of action, being acid-activated prodrugs and covalently bind cysteine residues of the gastric H⁺/K⁺-ATPase. The compound accumulated in the acid space the secretory canaliculus of the stimulated parietal cell and consequently converted to the active form, a cationic thiophilic reagent. These activated species binds covalently to one or more cysteines accessible from the luminal face of the pump, interfering outflux of hydronium ion from cytoplasm. Similar considerations applying to design of compounds substituting K⁺ on the outside surface led to the development of acid pump antagonists (APAs) [12–14]. Whereas the PPIs have a unique targeting and covalent inhibitory action on the proton pump based on their chemistry and the biology of the parietal cell, another class of compound, APA, have a structural specificity for their target region on the pump, close to the K⁺ binding region of the H⁺/K⁺-ATPase and are, thus, K⁺ competitive and dissociate from the pump when their blood concentration drops. Therefore, this agent have great advantages in terms of independent of secretory status, no lag time required, reversible in actions, and could be therapeutic antacids, allowing “on-demand dosage”.

Recently, we have found that the H⁺/K⁺-ATPase inhibitors...
tubulovesicle to the membrane of the secretory canaliculus where it is activated.

The gastric acid pump, p-type H⁺/K⁺-ATPase

The gastric acid pump, p-type H⁺/K⁺-ATPase, on the parietal cell consists of two subunits, a 114 kDa α-subunit and a 35 kDa β-subunit [21, 22]. The α-subunit containing ATP and cation binding sites carries out the catalytic and transporting function of the proton pump. The heavily glycosylated β-subunit is required for endocytic retrieval of the H⁺/K⁺-ATPase from the canalicular membranes and is also essential for protecting proton pump from environment of acid milieu. The α-subunit is shown with ten transmembrane segments, a large cytoplasmic molecular mass for binding and interacting with ATP. The β-subunit composes a single transmembrane segment, a relatively short amino-terminal cytoplasmic segment and a longer extracellular domain including sites for glycosylation and three stabilizing disulfide bonds (Fig. 2). Interactions between the α- and β-subunits occur within the membrane and at the extracellular locus to stabilize functional activity and conformational status of the holoenzyme. Binding of K⁺ ion at its respective extracellular and intracellular sites is known of influence subunit interaction and protein conformation. The extensive glycosylation through seven N-linked oligosaccharides may serve an important function in protecting the enzyme from the destructive extracellular environment.

Kinetics of the H⁺/K⁺-ATPase have defined the reaction steps showing three major conformations, with ion-binding sites facing inward [the E1 conformation], ion sites occluded [the occ conformation], and ion-binding sites facing outward [the E2 conformation]. The transition between these conformations is driven by ATP binding, phosphorylation, and dephosphorylation. Transport-catalysis coupling in the gastric H⁺/K⁺-ATPase, in which hydronium ions (H₃O⁺) and MgATP bind to the cytoplasmic face of the enzyme [the E1 conformation], and then the enzyme is phosphorylated, moving the hydronium ion into the membrane domain and the out to the exoplasmic face [the E2 conformation]. After release of the hydronium ion, in the presence of K⁺ ion extracellularly enabled by the KCl channel in the canalicular membrane, K⁺ ion binds to the outward conformation of the phosphorylated pump. The K⁺ ion is transported inwardly during the dephosphorylation step. In the absence of K⁺ ion, the pump stops in the E2P conformation [23, 24].

Acid suppression through gastric pump inhibitors; PPI and APA

Gastric acid is pathogenic in many gastrointestinal disorders, such as gastroesophageal reflux disease and peptic ulcer disease. Treatment for acid-related disease has been revolutionized by drugs that significantly suppress gastric-acid secretion. Histamin-2-receptor antagonists (H₂RA)
have been used extensively for acid suppression for more than 25 years. Subsequently introduced proton pump inhibitors (PPIs) provide more potent acid suppression and greater clinical efficacy than H2RA. PPIs are considered the drugs of choice for acid-related diseases including gastroesophageal reflux diseases (GERD) and peptic ulcer diseases. Regimens consisting of a PPI plus 2 or more antimicrobials are also used for the eradication of *Helicobacter pylori*, a cause of PUD [25, 26]. Five PPIs are approved by the US Food and Drug Administration (FDA)-omeprazole, lansoprazole, omeprazole, pantoprazole and rabeprazole (Fig. 3A).

PPIs are substituted 2-pyridyl methyl-sulfiny benzimidazoles that share a similar core structure. These agents are protonatable week bases with pKa values of ~4. Therefore PPIs accumulate selectively in acidic spaces with a pH <4, which are found primarily in the secretory canaliculus of gastric parietal cell. In this acidic environment, protonation of pyridine and benzimidazole nitrogens results in formation of a tetracyclic sulfonamide, which represents the active form of the drug. The sulfenamid binds to exposed cysteine residues in the α-subunit of H+/K+-ATPase to form covalent disulfide bonds, which inhibit the activity of the pump. Because PPIs bind covalently, the duration of action of these drugs extends beyond their plasma half life of ~1 to 2 hours. Gastric acid secretion is restored by translocation of new H+/K+-ATPase to the secretory canaliculus membrane [27]. Because the PPIs have a covalent inhibitory action on the proton pump based on their chemistry and the biology of the parietal cell, there is lag time expected.

Recently another class of compound, acid pump antagonist (APA), is a reversible inhibitor of gastric H+/K+-ATPase, which competitively binds with the luminal K+ ions to their binding site of the proton pump and dissociates from the enzyme when their blood concentration falls. Since the inhibitor discriminates the K+ affinity site of H+/K+-ATPase from that of Na+/K+-ATPase, it has little effect on Na+/K+-ATPase. In contrast to the PPIs, they are able to inhibit the pump without acid activation, but because of property of its week bases, they are accumulated in the canaliculus of the parietal cell. The K+-competitive acid pump antagonists such as the SCH28080, BY841, MeDAZIP+, and MDPQ are bind noncovalently and their specific site of attachment is much harder to predict because the region of the protein that binds K+ or whose conformation prevents K+ binding is not known (Fig. 3B). The binding can be investigated by mutational analysis or by generating photoaffinity derivatives. The photoaffinity derivative thereof, MeDAZIP+ was shown to be K-competitive and was covalently bound to the TM1/TM2 domain of the H+/K+-ATPase. Substitution in the loop between TM1 and TM2 domain of the enzyme did not affect the inhibitions, resulting in the conclusion that inhibition depends on interaction of the inhibitor with the TM1/TM2 membrane domain itself rather than with connecting loop. Mutations have also shown that the loop between M5 and M6 is an important determinant of binding of SCH28080 and the binding sites for K+ and the inhibitor are different and that competitive kinetics arises from mutual exclusion of binding. In contrast to PPIs, the action of APAs is independent of the secretory status, and there is no lag time expected. Therefore, these are fast-acting compounds able to abolish acid secretion during their presence in the

![Fig. 3](image_url) The structures of proton pump inhibitors (PPIs) and acid pump antagonists (APAs) The core structure of all the clinically approved PPIs is timoprazole and K+-competitive APAs showing the core SCH28080 with photoaffinity derivatives.
blood. These drugs 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. However, they are not introduced into clinical practice, yet.

Revaprazan (Revanex®), 5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinoline-2-yl) pyrimidine hydrochloride, is a novel APA that is currently approved by the Korea Food and Drug Administration (KFDA) as new drug for the treatment of gastric diseases including peptic ulcer disease (Fig. 4A and B) [28, 29]. This APA presents a reversible mode of action against H⁺/K⁺-ATPase, which is distinct from that of PPIs known for an irreversible inhibition (Fig. 4D). The characteristics of the drug have great advantages in aspect of inhibition of gastric acid secretion rapidly as fast on-set action and effectively without sustained hypergastrinemia. This inhibitor is competitive with the luminal K⁺ ions and shows a highly selective affinity for H⁺/K⁺-ATPase, but displays low affinity to Na⁺/K⁺-ATPase (Fig. 4C). Therefore it is expected that revaprazan would be a promising therapeutic agent for the treatment of gastric acid-related disease.

**APA beyond Acid Suppression; Another Potential Application for Gastric Inflammatory Disease as well as Cancer**

As mentioned before, anti-secretory drugs had been extensively applied for the treatment of acid-related diseases. Also, regimens consisting of a PPI plus 2 or more antimicrobials are also used for the eradication of *Helicobacter pylori*. Recently, we found that beside of the anti-secretory action, the H⁺/K⁺-ATPase inhibitors can regulate intracellular signaling pathways to apoptosis as well as...
inflammatory response. Following treatment of pantoprazole among PPIs, apoptotic cell death was seen selectively in gastric cancer cells, never seen in same cultured non-cancerous cell lines, accompanied with MAPK ERK1/2 deactivation. As for these selectivities of PPI’s apoptotic activity, normal gastric mucosal cells showed the resistance to PPI-induced apoptosis through by the overexpression of anti-apoptotic regulators including HSP70 and HSP27. In a xenograft model of nude mice, administration of the PPI significantly inhibited tumorigenesis and induced large-scale apoptosis of tumor cells. These data suggest that proton pump inhibitors (PPIs) could be extensively used for selective anti-cancer effects.

In addition, conditioned media from *H. pylori*-infected gastric epithelial cells directly induced a tubular formation of HUVEC cells and the increase of *in vitro* angiogenesis was suppressed by PPI treatment. Infection of *H. pylori* significantly up-regulated expression of HIF-1α and VEGF in gastric epithelial cells and the expression of proangiogenic factors was mediated by MAPK activation and partially responsible for NF-κB activation. PPI effectively inhibited the phosphorylation of MAPK ERK1/2 that is a principal signal for *H. pylori*-induced angiogenesis. The fact that PPI could down regulate *H. pylori*-induced angiogenesis shed light on that anti-angiogenic treatment using PPI could be a promising protective therapeutic approach for *H. pylori*-associated carcinogenesis.

Interestingly, revaprazan (Revanex®), one of the first APA completed trials for clinical use and approved by Korea FDA as new remedy for gastric diseases including duodenal ulcer and gastritis, displayed a potent anti-inflammatory action on both the NSAID-induced acute gastritis and the EtOH-induced acute gastritis animal model. Pretreatment of the APA completely inhibited NSAID (indomethacin) or EtOH-induced gastric injury (Fig. 5). The effect of the APA might be induced by inhibiting the MAPK or NF-kappa B which is an executive critical enzyme in inflammatory response signaling. *In vitro* cell culture system, revaprazan has a strong inhibitory activity on *Helicobacter pylori*-induced NF-κB transcriptional activation. The suppression of NF-κB by the agent also documented in different damage factors such as H₂O₂ and EtOH. MAPK plays a critical role in inflammatory response as well as cell proliferation. Revaprazane like as pantoprazole shows a regulatory role in MAPK ERK1/2 signaling provoked by *Helicobacter pylori*. In additional, this APA has been revealed to cytoprotect gastric epithelium from cell injury provoked by several damages such as alcohol or NSAIDs. Pretreatment of revaprazole prior to alcohol expose significantly rescued gastric epithelial cells from the ethanol-induced cell death (Fig. 6A). The cytoprotective effect of this agent might be come from the induction of proteins of which function was well known as cytoprotection. Twenty five micromole of revaprazole stimulates expressions of the cytoprotective heat shock proteins like as HO-1, HSP27, and HSP70 as well as cytoprotective heat shock proteins like as IL-8, VEGF, and COX-2 (Fig. 6B and C). All together, our results shed bright light on speculation that gastric proton pump inhibitors including APA could be considered as wide spectrum of protective therapeutic approach for inflammatory disease as well as acid suppression. In addition, since APA could be effective so fast in acid suppression, it could be right drug for “on demand therapy” based on the action of therapeutic antacid.

---

**Fig. 5.** Anti-inflammatory effect of revaprazan on both the NSAID-induced acute gastritis (A) and the EtOH-induced acute gastritis animal model (B). Six week-old Sprague-Dawley male rats are administrated with 10 mg/kg revaprazan via oro-gastric tube prior to exposure to either indomethacin (A, 40 mg/kg for 16 hrs) or absolute ethanol (B, 6 ml/kg for 1 hrs). Gross findings showing the gastric mucosal lesions in rat exposed to NSAID or EtOH significantly reduced in rat pre-administrated with revaprazan. Since the major principal actions of mucosal injuries were not related top gastric acidity, that is, direct toxicity, gastric inflammatory mediators, and oxidative stress related, APA might impose the anti-inflammatory and gastroprotective actions as well as acid inhibition.
Conclusions

More thorough researches regarding the exact secretion mechanisms in the canaliculi of parietal cells can open the more deep insights of the significance and implication of ABC (ATP binding cassette) regulation, endowing the more complex biological myth of acid secretion and its inhibition. The recent achievement of the development of novel APA comprises of the meaning that more rapid and reversible control of acid secretion was coming true, fulfilling “on demand therapy” in case suffering from considerable heartburn or dyspepsia, and providing the immense application for gastric inflammatory diseases not related to acid. However, more detailed study will be required to draw the more conclusive evidences for intense clinical application or another extensive mode of pharmacology beyond acid suppression.

Acknowledgment

This study was supported by a grant of The Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (01-PJ10-PG6-01GN14-0007).

References


