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Proton pump inhibitors are now massively utilized medications worldwide but their H^+,K^+-ATPase and non-H^+,K^+-ATPase mediated side effects are poorly appreciated by both clinicians as well as researchers.

Proton pump inhibitors: actions and reactions

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Proton pump inhibitors are the second most commonly prescribed drug class in the United States. The increased utilization of PPIs parallels the rising incidence of reflux disease. Owing to their clinical efficacy and relative lack of tachyphylaxis, PPIs have largely displaced H-2 receptor antagonists in the treatment of acid peptic disorders.The elevation of intragastric pH and subsequent alterations of gastric physiology induced by PPIs may yield undesired effects within the upper GI tract. The ubiquity of the various types of H^+, K^+-ATPase could also contribute to non-gastric effects. PPIs may influence physiology in other ways, such as inducing transepithelial leak.

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Overview of proton pump inhibitors

The market for proton pump inhibitors

Proton pump inhibitors (PPIs) are extremely popular and prevalent medications. Owing to their proven efficacy and safety, they are frequently prescribed both in the hospital and on an outpatient basis. These drugs consist of omeprazole (Prilosec), lansoprazole (Prevacid), pantoprazole (Protonix), rabeprazole (Aciphex), esomeprazole (Nexium), and tenatoprazole. PPIs are used to treat acid-related diseases, including gastroesophageal reflux disease (GERD), Barrett's esophagus, peptic ulcer disease (PUD), Zollinger-Ellison syndrome, gastrinomas, and esophagitis/gastritis (inflammation of the esophagus/stomach) [1]. Non-invasive pharmacological approaches to these conditions are far preferred to surgical intervention.

GERD is a syndrome characterized by the reflux of gastric acid into the lower esophagus often inducing chest or epigastric pain or burning [2]. These symptoms are often confused with cardiac chest pain, prompting large numbers of emergency department visits [3]. Up to 40% of the Western population complains of these symptoms, classically identified as heartburn [4]. Initial treatment recommendations include lifestyle modifications, cessation of smoking and weight loss. Nonetheless, pharmacological therapy is frequently instituted, resulting in an enormous

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number of PPI prescriptions annually. PPIs have revolutionized the treatment of GERD by suppressing acid production and, therefore, its reflux into the esophagus, providing symptomatic relief [5]. GERD is a chronic disease, and its management with PPIs helps to heal erosive esophagitis and slow or halt progression to Barrett's esophagus, and potentially adenocarcinoma [4].

PUD can range from superficial mucosal erosion to hemorrhagic ulceration of either the stomach or the duodenum. The most common causes of PUD today are the use of non-steroidal anti-inflammatory drugs (NSAIDs) and the bacteria *Helicobacter pylori* (*H. pylori*). Idiopathic ulcers not due to NSAIDS or *H. pylori* are also becoming increasingly common [6]. Rarer causes include Zollinger-Ellison syndrome or other hypersecretory states, where there is an inappropriately elevated release of gastric acid secondary to a gastrin-secreting tumor. PPIs promote gastric and duodenal mucosal healing in PUD by decreasing the acid load in this highly acidic environment. They also provide synergy in the eradication of *H. pylori* when administered in conjunction with amoxicillin and clarithromycin, referred to as 'triple therapy' [7]. Bleeding peptic ulcers are a major source of mortality and morbidity, especially among the elderly and patients being treated with anticoagulants or platelet inhibiting therapy. Trials have shown that PPIs are the preferred treatment by successfully achieving hemostasis via raising intragastric pH, ultimately allowing a clot to form and stabilize [8]. This therapy is usually administered via continuous intravenous infusion of a PPI.

Gastrointestinal symptoms are a frequent reason for presentation to a physician [9]. By the mid 1990s, the use of PPIs increased fourfold among general practitioners [10]. Also, patients admitted to the hospital are frequently prescribed PPIs and subsequently continue on such medication upon discharge [11]. This begins in the hospital as a method of stress ulcer prophylaxis. Stress ulcers can develop in the crucially ill owing to splanchnic arterial hypoperfusion, leading to mucosal ischemia, altered mucosal defense, and finally gastric mucosal damage [12,13]. There is a significant risk for the development of stress ulcers in patients requiring mechanical ventilation for more than 48 h and in patients with coagulopathies, defined as an international normalized ratio (INR) >1.5, a prothromboplastin time (PTT) >2 times normal, or a platelet count <50 K [12,14]. Nevertheless, studies show that stress ulcer prophylaxis is generally overused in low risk hospital inpatients [15,16]. Unfortunately, this excessive and unwarranted use of PPIs often persists well after hospital discharge. This issue of potential PPI overuse is discussed further on in this review.

GERD is a very common complaint in pregnant females. This is due to a decrease in lower esophageal sphincter tone induced by the hormonal changes of pregnancy, coupled with an increase in intra-abdominal pressure from an enlarging uterus and displaced abdominal organs [17]. PPIs are also prescribed to pregnant females with increasing frequency. Omeprazole is classified by the FDA as a category C drug owing to fetal mortality noted in animal studies (see Prilosec package insert). The FDA received reports of birth defects in humans following omeprazole exposure during pregnancy, but multiple case reports did not identify this risk [18,19]. A subsequent meta-analysis noted a non-significant relative risk for birth defects in humans with the use of omeprazole, but the FDA maintains its category C rating [20]. The newer PPIs, classified as category B, have proven safe in animal studies [20]. Studies comparing pregnant women receiving PPI therapy to controls have failed to demonstrate an elevated risk of teratogenicity, low birth weight, or preterm deliveries [21,22]. Although PPIs provide symptomatic relief to pregnant women, GERD generally subsides following fetal delivery, obviating the need for ongoing acid suppressive therapy.

GERD is very common in the pediatric community and a frequent reason for visits to the pediatrician [23]. There is a higher prevalence in infants than children, thought to be a result of immaturity of the esophagus and stomach, higher liquid intake, and potential genetic factors, usually resolving between one and three years of age [24]. Positioning while sleeping, thickening of formula, and other lifestyle changes are recommended as first line treatment for infants. If symptoms continue, PPIs are thought to be both effective and safe to use in infants and children, having been studied with >one year of continued therapy [25,26]. As in adults, PPIs are rapidly absorbed in children, reaching a maximum plasma concentration 1–3 h after oral administration, but are cleared more quickly in children owing to an increased metabolism [27]. Nevertheless, they are efficacious without impeding the growth and nutritional status of infants and children.

Further in the review a more complete picture of 'side effects' of PPIs is provided. Before we get to that point, an important distinction needs to be made. The 'intended (desired) effect' of a PPI is to inhibit acid release into the stomach lumen by means of directly inhibiting the gastric H^+,K^+-ATPase. A true 'side effect' of a PPI would be to produce an unintended effect that proceeds via a target other than gastric H^+,K^+-ATPase. This would probably include, for example, cytochrome P450 inhibition (and resulting drug clearance inhibition) by PPIs. However, there are other unintended effects of PPIs that derive not from a non-H^+,K^+-ATPase target, but instead proceed secondarily from the inhibition of the gastric H^+,K^+-ATPase itself. The best example is hypergastrinemia that results from the elevated pH of gastric luminal contents. The morphological effects of PPIs on the gastric mucosa might also be traced to here. Still other effects however, such as the newly described PPI-induced transmucosal gastric leak, are simply unknown regarding their mechanism and the target molecule involved.

One other introductory caveat is that when one sees a reference to an effect caused by a given PPI, it does not imply that all PPIs will produce this effect (nor conversely does it imply that the effect is specific to that PPI only). Published studies frequently utilized only one PPI when reporting an effect. It is not correct to infer therefore that, for example, lansoprazole will cause an effect just because omeprazole does (or vice versa). Very different degrees of inhibition of different PPIs on liver cytochromes are in fact known to exist.

Finally, there is the issue of whether the given molecular interactions or cellular 'side' effects of specific PPIs we report here have actual clinical ramifications. In many cases we definitely report that they do (hypergastrinemia, fundic polyposis, malabsorption issues, bacterial overgrowth, etc.). In other cases, especially where the study we report was performed in cell culture or isolated tissue models, a direct clinical repercussion cannot be claimed from the data presented. In those cases one needs to view the effect as a case of the 'jury is still out' and not assume that a clinical effect is or is not attendant, but that simply more study is needed. Attention

likewise needs to be paid to the concentration of PPI that is used to produce an effect in an *in vitro* study, and ask oneself if this level of drug is ever likely to occur *in vivo*.

The potential overprescription of PPIs

As stated above, PPIs are one of the most frequently prescribed classes of medication. A growing literature is focusing on their potential overuse in the outpatient and in the hospital setting ([28] [29] [30]). In 2006, expenditure on PPIs reached seven billion dollars globally. According to an editorial in the *British Medical Journal* in 2008, between 25 and 70% of patients taking PPI's have no appropriate indication, with potentially two billion dollars each year being spent unnecessarily on these medications. In many instances a disregard for prescribing guidelines by local practitioners is responsible for this monetary indiscretion. For instance, a prospective audit of a series of patients admitted to a hospital in Wales demonstrated that 25% of patients were taking a PPI. However, only half of these patients had an indication for their use. A repeat audit was conducted six months after the National Institute for Health and Clinical Excellence guidelines for prescribing PPI's were put forth. Once again, the same proportion of admitted patients were taking PPI's and only half of these had a recommended indication [31].

Overprescribing of PPIs is not only occurring in the primary care setting but also in the secondary care setting. In Ireland, for instance, a study found that 33% of hospitalized patients taking PPI's did not meet their country's criteria for use of these medications. That number rose to an astounding 67% in a study conducted on hospitalized patients in the UK who were taking PPIs. Compounding the problem, many patients are continuing to take PPIs upon hospital discharge for an unnecessary amount of time because the suggested length of treatment is not specified on the discharge instructions. In one UK center, less than 20% of hospital discharge instructions included the suggested length of treatment. Further, only half of the discharge instructions specified why the drug was initiated in the hospital [31].

Structure and function of PPIs

PPIs consist of either substituted pyridylmethylsulphonyl benzimidazole or imidazopyridine derivatives, all products of the original structure for the oldest PPI, omeprazole (Figure 1) [32]. These antisecretory drugs inhibit the gastric H^+, K^+-adenosine triphosphatase (ATPase), the gastric proton pump, by covalent binding to cysteine residues of the ATPase in the parietal cell [33]. PPIs have a pK_a of 4.0, causing the medication to accumulate in the acidic secretory canaliculus through pyridine protonation by stimulated parietal cells [7]. Within the canaliculus, they are structurally modified by the high acid content, converting the PPI prodrug to its active sulphenamide form, followed by irreversible binding to the proton pump, inhibiting acid secretion [32]. PPIs are associated actually with *two* pK_a values, a primary pyridine pK_a of 4.0 allowing for accumulation in the canniculus of the parietal cell, and a secondary benzimidazole pK_a of 1.0 involved in acid activation of the PPI [34]. The secondary pK_a of rabeprazole is approximately 5.0, and therefore, can be activated at a higher pH than other PPIs [35].

PPIs made up of an imidazopyridine ring are activated more slowly than those with a pyridylmethylsufinyl benzimidazole ring, but are more resistant to reversal [33]. The different preparations have an average half-life of about 60 min, ranging from 30 min to 2 h, but continue to elicit an effect for about two to three days [7]. This is due to the covalent bonding of the active sulphenamide form of the PPI to the ATPase pump [34]. Despite the small variations among the different drugs, their efficacy remains alike [36].

PPIs are metabolized through the liver via the cytochrome P450 (CYP) 2C19 pathway, as described below, thus creating the potential for altering the plasma concentrations of co-administered medications that are also metabolized by the cytochrome P450 system. Care should be taken when prescribing such medications that need to be titrated within narrow therapeutic windows, such as the anticonvulsant, phenytoin, and the anticoagulant, warfarin [37]. Genetic polymorphisms in the CYP2C19 can cause variations in the clearance of PPIs, ultimately increasing their plasma levels [38]. These topics are discussed below in more detail (Figure 2).

Histamine 2-receptor antagonists (H2RA) (Tagamet, Zantac, Pepcid, etc.), the 'previous generation' of drugs for GERD and acid-peptic disorders, act on the Histamine 2 receptors of gastric parietal cells. Histamine, along with acetylcholine and gastrin, activates acid secretion by parietal cells. Blocking the final common pathway of acid secretion at the H^+,K^+- ATPase pump, PPIs are more potent acid suppressants than H2RA, which block only one

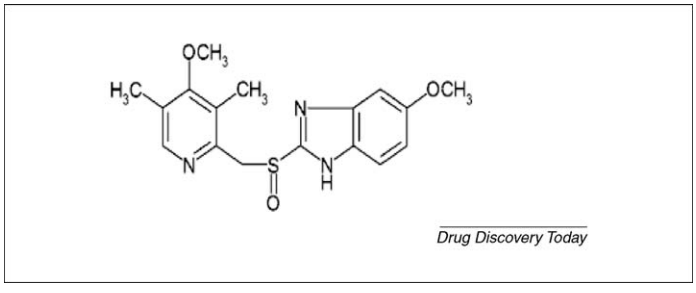

FIGURE 1

Molecular structure of omeprazole.

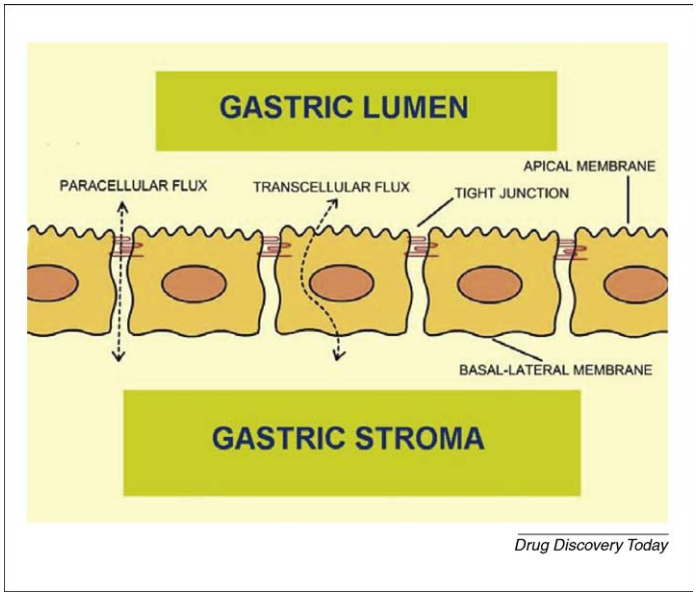

FIGURE 2

Considerations in gastric transepithelial permeability.

of the three receptor-mediated pathways of acid secretion [39]. PPIs have a more dramatic result on intragastric pH, generating significantly higher luminal pH values than H2RA. They have also been found to be more effective in preventing persistent or recurrent bleeding from a peptic ulcer [8,40]. Tachyphylaxis is a greater issue for H2RA [39]. Concerning safety issues, there has been a link to an increase in nosocomial pneumonia with the use of H2RA while on mechanical ventilation, thought to be a result of retrograde colonization of bacteria in the pharynx secondary to overgrowth in the stomach [41,42]. Cimetidine, an H2-antagonist, has a greater effect on the cytochrome P450 pathway than PPIs [39]. Hematologic toxicity with thrombocytopenia has also been attributed to H2-antagonists [12].

The (gastric lumen) pH response to PPI use is variable and dependent upon route of administration along with dosage and timing of administration related to meals. In order to evaluate the pH accurately, either an intragastric pH probe must be used to assess the gastric efficacy, or a 24 h intraesophageal pH monitor can be utilized to determine the drug effect within the esophagus [43,44]. Ideally, the intraluminal pH should be above 4.0 with regular recommended oral use and greater than 6.0 with continuous intravenous use. Low dose treatment with omeprazole at 20 mg orally once daily has been shown to produce a rise in intraluminal pH over 4.0 for more than 8 h in the majority of patients [45]. An intravenous infusion of a PPI is used for clot stabilization in bleeding ulcers, frequently keeping the intraluminal pH above 6.0 [43]. Nonetheless, resolution of symptoms in GERD patients does not mean that the pH has risen appropriately, which can allow persistent pathological acid reflux in patients with Barrett's esophagus [46]. Both intragastric and intraesophageal pH control has been found to be more difficult in patients with Barrett's esophagus compared to those without the condition [44]. PPI therapy can be adjusted by increasing the frequency of use per day or the amount taken with each dose.

Morphological side effects of proton pump inhibitor use

Chronic suppression of gastric acid with PPIs is generally well tolerated, with few clinically evident side effects. However, multiple investigations in both humans and animal models have exhibited both histological and gross anatomic changes induced by inhibition of the proton pump.

PPI effects on gastric exocrine cells

The physiology of gastric acid secretion relies on the complex interplay of multiple hormones, such as gastrin, somatostatin, and histamine, as well as neurotransmitters, namely acetylcholine [47]. The inhibition of acid secretion by a PPI interrupts the balance of these hormones. Gastrin, the most potent stimulant for the secretion of gastric acid, has been shown to be present at elevated levels in serum following initiation of PPI therapy [48]. A study in humans by Lamberts *et al.* showed an increase in the fasting serum gastrin level in chronic PPI users after three months, with no additional increase in levels with prolonged therapy up to 10 months [49].

Parietal cells, which are responsible for gastric acid production, have been observed to increase in size following long-term (>12month) use of omeprazole [50]. In this study, patients receiving omeprazole for greater than 12 months exhibited (in electron micrographs) greater parietal cell mass, height, and number compared with those receiving either ranitidine, no acid suppression, or omeprazole for less than 12 months. An earlier investigation utilizing shorter courses of PPI therapy (two to six weeks) showed no difference between cell populations or mucosal thickness on biopsy specimens taken before and after therapy [48].

A study by Kakei *et al.* exhibited a decrease in the number of chief cells in the gastric mucosa after administration of omeprazole to rats for 28 days [51]. The authors also noted an increase in immature pepsinogen-producing cells expressing class III mucin, concomitant with a decrease in the mucosal levels of both pepsinogen and its mRNA. These changes were observed at doses that induced acid suppression, and all findings were reversible following cessation of PPI therapy.

PPI effects on gastric endocrine cells

The endocrine cells of the stomach, namely, the enterochromaffin-like (ECL) cells, D cells, enterochromaffin (EC) cells, and A-like cells have also been investigated during PPI therapy. ECL cells, located in the base of oxyntic glands, are responsible for the synthesis and release of histamine, another stimulant of gastric acid secretion. Gastrin acts upon CCK-2/gastrin receptors to directly induce ECL cell division [47]. In a rodent study, administration of omeprazole to rats, guinea pigs, and hamsters for two to ten weeks was associated with an increase in ECL cell size, with maximal effect seen after two weeks, and no further increase in size noted after four weeks [52]. Furthermore, an increase in the size of cytoplasmic vesicles, thought to represent the storage site of secretory products, was noted with omeprazole. A study in humans showed a doubling in argyrophilic cell (ECL cell) volume in patients after five years of PPI therapy [49].

Significant concern arose in the early 1980s when oncogenicity studies revealed an increased rate of carcinoid tumors of ECL-cell origin in rats after life-long acid suppression with omeprazole [53,54]. Fortunately, human studies utilizing omeprazole [55] and lansoprazole [56] did not demonstrate an increased risk of carcinoid formation in patients undergoing long-term acid suppression. It has also been shown that the elevated gastrin levels associated with PPI use did not seem to stimulate colon tumor growth in mice receiving subcutaneously implanted tumors [57].

Other endocrine cells, namely the ghrelin-producing A-like cells and D-cells, which release somatostatin, did not exhibit hypertrophy following long-term omeprazole therapy in rats [58].

Fundic gland polyps

The following unintended effect of PPIs is a good example of an effect resulting from the *desired* action of PPIs on inhibiting acid secretion and thereby elevating luminal pH and secondarily serum gastrin levels. Multiple studies have examined the correlation between PPI use and presence of fundic gland polyps. Jalving *et al* noted that long-term (>12 month) use of PPIs was associated with an increased risk of fundic gland polyps, while short-term (<12 month) use was not associated with this risk [59]. Biopsy specimens revealed a high frequency of parietal cell hyperplasia. Multiple case reports have noted development of fundic gland polyps following initiation of PPI therapy with resolution of polyps following cessation of therapy [60,61]. The increased prevalence of

fundic gland polyps has been noted in children on long-term omeprazole as well [62]. In contrast to the above studies, a large retrospective study of *H. pylori* negative patients noted nearly identical frequency of fundic gland polyps in PPI users *versus* control patients who were not taking PPIs [63].

Only one of 107 fundic gland polyps associated with PPI use exhibited dysplasia [59]. By contrast, nearly half of all patients with familial adenomatous polyposis had dysplastic gastric polyps in one study [64]. Exposure to acid-suppressive medications was associated with a lower prevalence of dysplastic fundic gland polyps. To date, there is no evidence that hypergastrinemia induces neoplasia; however, gastrin may elicit trophic effects on previously existing neoplastic cells that express its receptor [65].

Fossmark *et al*. undertook an evaluation of gastric pH, serum gastrin levels, and serum chromogranin A (an ECL cell marker) in PPI users with fundic gland polyps [66]. The study demonstrated no difference in these levels between PPI users with and without gastric polyps. All patients with polyps were *H. pylori* negative, while four of six PPI users without polyps were positive for *H. pylori*. Another study notes regression of fundic gland polyps in two patients following infection with *H. pylori* [67].

Atrophic gastritis

PPI therapy has also been implicated in the development of atrophic gastritis, specifically in patients infected with *H. pylori*. In an analysis of patients with reflux esophagitis treated with either omeprazole or fundoplication without subsequent PPI use, none of the fundoplication patients developed atrophic gastritis, regardless of *H. pylori* status [68]. In patients treated with PPIs, 18 of 59 patients with *H. pylori* developed atrophic gastritis, while two of forty-six who were not infected with *H. pylori* developed atrophy. This study was a comparison of two cohorts of patients, rather than a randomized, controlled trial. A similar study that randomized patients to acid suppression or anti-reflux surgery did not exhibit an increased rate of gastric atrophy in patients on long-term omeprazole [69]. A further, smaller study of *H. pylori*-positive patients in Japan also failed to show an increased rate of gastric atrophy in patients on long-term PPI therapy [70].

A subsequent trial of *H. pylori* positive patients on long-term omeprazole illustrated improvement of glandular atrophy and improvement of mucosal inflammation following *H. pylori* eradication, even with the continued use of a PPI [71]. The authors recommended eradication of Helicobacter in all patients who require long-term acid suppression.

There has been much conjecture regarding the possible migration of *H. pylori* from the gastric antrum to more proximal segments of the stomach, specifically the fundus and corpus, in patients taking PPIs. This migration, several authors have suggested, may help explain the persistence of *H. pylori* infection along with the gradual progression to chronic atrophic gastritis despite triple eradication therapy. Possible explanations have been that delivery of amoxicillin to the gastric fundus mucosa was poor, antibiotics were potentially less active in the acid-secretory cells of the gastric body and fundus, or that *H. pylori* was simply more resistant to treatment in this environment [72]. However, this suspected migration of *H. pylori* in response to PPI therapy has been disputed by Graham *et al*. [73], whose study of 72 patients with histologically documented *H. pylori* demonstrated that omeprazole was detrimental to the gram negative spirochete in both the antrum and the corpus. In that study, patients with *H. pylori* cultured from both antral and corpus biopsies were treated with omeprazole for four weeks. Repeat endoscopy with histologic examination was conducted at the conclusion of therapy and again four to six weeks after ending therapy. All 72 patients had *H. pylori* present in both the antrum and corpus at four to six weeks post-therapy. However, only 54% of patients had antral *and* corpus positive specimens at the conclusion of therapy. Further, the number of colonies of *H. pylori* per biopsy was significantly diminished in both the antrum *and* corpus at the end of therapy in those patients who did remain *H. pylori* positive.

Physiological and pharmacological side effects of proton pump inhibitors

The literature on PPI drugs is vast. Their safety and clinical actions are reviewed frequently and have been well characterized [1,26,33,36–38,74,75]. This present review of side effects of PPIs does not claim to be fully comprehensive, but strives to highlight a number of important issues related to unintended consequences of PPI use, and hold out for consideration whether these actions are, or are not, clinically significant. To begin, a working definition of 'PPI-induced side effects' needs to be formulated. For the purposes of this review, we consider it to be any effect of PPIs other than the desired effect of suppression of acid secretion. In considering very generally the types of 'side effects' that PPIs can generate, it helps to visualize the phenomenon in three broad classes:

1. effects arising from inhibition of parietal H^+,K^+-ATPase and elevated gastric lumen pH
2. gastric and non-gastric side effects stemming directly from H^+,K^+-ATPase inhibition
3. non-gastric side effects that seemingly do not stem from H^+,K^+-ATPase inhibition

Effects arising from elevated gastric lumen pH

The desired effect of H^+,K^+-ATPase inhibition in the stomach is to reduce secretion of acid in the stomach and elevate luminal pH. This H^+,K^+-ATPase inhibition probably leads to related effects on parietal cell bioenergetics, such as a decrease in mitochondrial enzyme activity [76]. This reduction of acid secretion allows for mucosal healing in GERD and other acid-peptic disorders. There are, however, numerous repercussions of elevating gastric lumen pH that are more on the order of side effects. First, by changing luminal pH (typically from as low as pH 1–2 to as high as pH 6–7 in a person taking PPIs) one may change the solubility of substances in the lumen (e.g. calcium salts) and/or change the form in which substances exist, ionized or un-ionized, such as protonated or unprotonated carboxyl groups. This should not be problematic in the stomach, where minimal absorption occurs, but elevation in duodenal luminal fluid pH may perhaps lead to more substantial alterations in absorption.

PPI use can associate with suboptimal iron absorption [77,78]. Patients with hemochromatosis required significantly less phlebotomy treatments annually when using a PPI. Further analysis showed that PPI use decreased the absorption of dietary non-haem iron, which supplies at least two thirds of iron requirements. Gastric hydrochloric acid, whose concentration is lower in

patients on PPIs, normally promotes iron absorption by reducing ferric iron to its soluble ferrous form [45].

There has also been increased risk of hip fracture with long-term PPI use, suggesting interference with calcium absorption [79]. Less obvious may be the effects on absorption of vitamins and other nutrients, which may exist in one form at pH 2 and another at pH 6.

In addition, the optimum pH for certain luminally secreted enzymes such as pepsin (whose pH optimum is two) now no longer exist in the lumen, so effects on digestion may also theoretically occur. This is even more pointed in the light of possible effects of omeprazole on pepsin secretion *per se* [80]. Elevation of gastric luminal pH has been noted to induce alterations in protein hydrolysis within the gastric lumen [81]. This situation would pertain to the duodenum as well, since as the 'recipient' of gastric luminal contents, it will thus be affected by gastric lumen pH.

In the gastric antrum, elevated pH causes an exaggerated release of the hormone, gastrin, leading to hypergastrinemia [66]. The intended effect of this increased gastrin release is to stimulate the parietal cells of the gastric corpus to increase acid production. As a trophic hormone, however, gastrin can elicit growth changes in cells and has been implicated in gastric wall thickening/parietal and ECL cell hyperplasia [49,50], reduction of pepsinogen-producing cells and chief cells [51,82] and gastric fundic polyposis, as well as the gastric carcinoid formation peculiar to rats [53]. Hypergastrinemia, arising as a secondary effect of PPI action has also been implicated in non-gastric cellular and tissue effects, such as decreased $Na^+/K^+/Cl^-$ cotransporter and Cl^-/HCO_3^- exchanger mRNA in colon [83] and Leydig cell hyperplasia in rat testes [84].

A third consequence of elevated luminal pH would be to alter the pH of the gastric mucosa to a level that is optimal for colonization/growth of bacteria. This can of course result in alteration of the types and number of bacteria colonizing the upper GI tract, a phenomenon known to occur with PPI use [85–87]. The fear has been that the less acidic environment will lead to bacterial overgrowth and possible complications, such as enteric infections, malabsorption, or cancer [88,89]. Bacterial overgrowth has been demonstrated, but few important clinical outcomes have been linked to this finding. The change in flora may ultimately contribute to malabsorption of certain nutrients [90,91]. Vitamin B_{12} concentration has been found to be decreased with long-term acid suppression, and therefore, it can be argued that levels should be monitored with extended PPI use [92]. Lactobacillus and oral flora are affected with PPI use and changes in pH, but no consequences have been associated with this finding as of yet [93].

Effects on parietal and non-parietal cells probably stemming from direct H^+,K^+-ATPase inhibition: Tissue-specific differences in and distribution of H^+,K^+-ATPase

Certain side effects of PPIs derive from action on non-gastric epithelia in the absence of gastric cells or tissue. For example, an effect of PPIs on cardiac muscle cells in cell culture would fit this criterion. Similarly, tissue level studies with isolated liver parenchymal slices or isolated colonic mucosal sheets would also fit the model. Any effects of PPIs on these isolated cells or tissues must be primary effects because a stomach is physically removed from the picture. Conversely, in whole animal studies or clinical human studies it would be much more difficult to assign an effect as either primary or secondary.

For consideration of side effects of PPIs stemming from inhibition of H^+,K^+-ATPase in non-parietal cells, it is important to first realize that not all H^+,K^+-ATPases are the same and that noteworthy similarities exist between H^+,K^+-ATPases and Na^+,K^+-ATPases. Numerous excellent reviews of H^+,K^+-ATPase, including the issue of H^+,K^+-ATPase heterogeneity, have been published [94–99]. The H^+,K^+-ATPase belongs to the larger group of P type ATPases that share the common feature of having a phosphorylated intermediate, something not seen in the three other types of transport ATPases that include the structurally complex F and vacuolar H^+ ATPases, and ubiquitous ABC ATPases [100]. The P type ATPases mediate cation transport including Na^+, K^+, H^+, Mg^{++}, Ca^{++}, and Cu^{++}. The H^+,K^+-ATPase is structurally quite similar to the Na^+,K^+-ATPase with over 60% homology on the amino acid level, compared to Ca^{++}-ATPase to which it is only about 25% homologous [101]. H^+,K^+-ATPase and Na^+,K^+-ATPase are both composed of an α subunit that hosts the catalytic function and a β subunit that plays a role in stabilization and targeting of the enzyme to the plasma membrane and possibly K^+ transport [102]. They are the only P type ATPases to possess a β subunit [94]. H^+,K^+-ATPase exists as an oligomeric dimer [αβ]2 where one αβ complex is in the E1 conformation binding cytoplasmic ions and the other is in the E2 conformation binding luminal ions [101]. Na^+,K^+-ATPase does not share this feature.

Four isoforms of the α subunit of H^+,K^+-ATPase have been identified. Although all isoforms exhibit high homology at the amino acid level (60-87%), they are encoded by different genes and have a number of functions in different tissues [102]. Though initially named for the tissue in which they were first described, the isoforms were found to not be tissue specific.

HKα1 is responsible for gastric acid secretion and is the intended target of the PPIs. HKα1 has also been described in the cortical and medullary collecting ducts of the human kidney where it is thought to play a role in K^+ conservation with some evidence of increased levels of mRNA in the renal cortex in response to chronic hypokalemia [103].

HKα2 has been described in the distal colon, kidney, prostate, uterus, and heart. HKα2 is present at high levels in the distal colon where it participates in K^+ conservation and is thought to be integral in maintaining whole body K^+ levels, particularly during growth and development. Rat studies have shown HKα2 mRNA levels in the distal colon go from undetectable before birth to abundant in one and eight day old rats, and then subside progressively through adulthood [103]. Studies on the effects of omeprazole on HKα2 in the distal colon have been inconclusive but any evidence of disruption of K^+ conservation could have serious implications on PPI use in the pediatric population [104,105]. Inhibition of H^+,K^+-ATPase in the colon has been proposed as a cause of microscopic colitis that has been linked to lansoprazole use. This adverse reaction has only been described with lansoprazole and not other PPIs with the reason for this exclusivity still unclear [106]. HKα2, in the renal connecting tubules and the cortical, inner medullary, and outer medullary collecting ducts, has a central role in K^+ conservation demonstrated by increased levels of HKα2 mRNA and protein expression seen in chronic hypokalemia [103]. Experiments with HKα2-deficient mice do not show any abnormalities in K^+ homeostasis when fed a normal diet, but with a K^+ deficient diet they do exhibit

inappropriate renal and colonic K^+ losses [103]. HKα2 may also participate in Na^+ regulation as experiments on *Xenopus* oocytes using HKα2 cRNA demonstrated that H^+,K^+-ATPase can be active in an H^+-K^+ exchange and an Na^+-K^+ exchange mode [107]. HKα2 in the distal colon has been shown to be upregulated by dietary Na^+ depletion and aldosterone [107]. H^+,K^+-ATPase in rat myocardium participates in intracellular H+ and K+ homeostasis [108]. H^+,K^+-ATPase in the rat prostate contributes to prostate fluid acidification [109].

HKα3 has been noted in toad bladder and toad kidney but it is unclear if it exists in higher vertebrates. HKα4 is found in human skin, renal cortical and medullary collecting ducts, and in the brain. The functional roles of the HKα3 and HKα4 isoforms have not been as well defined.

There is only one identified H^+,K^+-ATPase β subunit, HK β-gastric, and three Na^+,K^+-ATPase β subunit isoforms, NKβ1, NKβ2, and NKβ3. HK β-gastric shares only 30–35% amino acid homology with the NKβ isoforms and is found only in the stomach where it pairs with HKα1 [110,111]. The Na^+,K^+-ATPase α subunit is also able to interact with the β-gastric subunit but this has thus far only been demonstrated *in vitro* [110]. Different β subunits can confer distinct functional properties such as different K^+ activation kinetics and this needs to be considered in cell types that express both H^+,K^+- and Na^+,K^+-ATPases such as gastric mucosa, distal colon, and renal collecting duct [110].

Before amino acid sequencing, the H^+,K^+-ATPase isoforms had been distinguished from one another by their sensitivity to inhibitors such as ouabain, a classic Na^+,K^+-ATPase inhibitor, and Sch-28080, a gastric H^+,K^+-ATPase inhibitor. The inhibitor binding sites are found on the α subunit and variations in the amino acids in these areas determine the isoforms sensitivity to the inhibitor [110]. HKα1 is sensitive to Sch-28080 but not to ouabain. HKα2 is sensitive to ouabain but only modestly inhibited by high concentrations of Sch-28080 [107].

PPIs are pro-drugs that require an acidic environment to be protonated, thus allowing them to bind to cysteine residues on the α subunit of the H^+,K^+-ATPase. The pro-drug can freely cross cell membranes until it is protonated rendering it less membrane permeable [33]. Non-gastric H^+,K^+-ATPases may lack the highly acidic compartment seen in the parietal cell canaliculus (an intracellular compartment in immediate contact with the H^+,K^+-ATPase) and may be unable themselves to change the PPI pro-drug to its active form. The excellent safety profile of PPIs has been partly attributed to these biochemical constraints on activation and the immobility of the activated drug. Studies by Puscas *et al.* [112] have suggested, however, that the activated drug is not as confined as previously thought. Also, though PPIs require a pH less than 2.5 for significant activation [101], activation can take place in less acidic environments, albeit at a slower rate [113]. It is, however, reported that PPIs can only accumulate in the acidic canaliculus of the parietal cell [7]. It is still possible that the H^+,K^+-ATPases outside the stomach and their role in electrolyte homeostasis and fluid acidification could be compromised by PPI use, though these effects have yet to be quantitated.

The ubiquitous nature of H^+,K^+-ATPase certainly makes it possible that inhibitors of H^+,K^+-ATPase may have effects in a wide array of cells and tissues. Interestingly, omeprazole is not very effective at inhibiting the vacuolar-type H^+-ATPase [114]. In addition, the colonic form of the H^+,K^+-ATPase may be immune from omeprazole action as well. Neither omeprazole nor Sch-28080 were very effective at inhibiting potassium transport across distal colon mucosa, suggesting the lack of a strong effect of omeprazole on the colonic H^+,K^+-ATPase [115]. Similarly, in purified apical membranes of distal colon, omeprazole did not inhibit H^+,K^+-ATPase activity, even though vanadate did [116]. Omeprazole and Sch-28080 were without effect on either potassium or proton flux across distal colon [104]. One study on guinea pig distal colon, however, did indicate omeprazole may have effect on colonic potassium transport [105].

Sch-28080 was surprisingly effective at inhibiting cochlear H^+,K^+-ATPase and caused dramatic reduction of endocochlear potential difference [117]. Omeprazole and Sch-28080 both inhibited pH recovery of renal collecting duct epithelia in response to addition and removal of NH_4Cl, indicative of omeprazole activity against the renal form of H^+,K^+-ATPase [118]. In a similar manner, omeprazole inhibited the formation of a pH gradient across the MDCK renal epithelium [119]. Effects of omeprazole on renal cell volume may also be a possibility [120]. An example of an even more profound biological effect on certain H^+,K^+-ATPase subtypes is the alteration of asymmetric gene expression in sea urchin development caused by omeprazole, lansoprazole, or Sch-28080 [121].

Presumably owing to their potent inhibition of the H^+,K^+-ATPase, PPIs have been observed to induce apoptosis or pro-apoptotic effects in certain cell types [122,123].This has not been completely verified owing to the imperfect correlation between H^+,K^+-ATPase abundance and apoptotic activity. Regardless of the exact mechanism, it does appear that PPIs can induce apoptosis under certain conditions. Considering that cancer cells can exist in an environment nearly devoid of blood flow, as well as at a low pH, research has been done to assess whether PPIs could be used as a possible therapy in the treatment of cancer, that is, can PPIs cause cell death or decrease cell viability of cancer cells. PPIs are thought to increase the susceptibility of cancer cells to chemotherapeutics owing to their effects on cellular pH, and have recently been shown to do so in cell lines derived from human melanomas, adenocarcinomas, and lymphomas [124]. Their pro-apoptotic activity - associated with production of reactive oxygen species, mitochondrial membrane depolarization, and chromatin condensation - may be the cause of the reduction in tumorgenesis *in vivo* and *in vitro* [122,125]. It has been well documented that PPIs reduce cancer cell viability and proliferation in a time- and dose-dependent manner while also reducing proton extrusion from the cell [123,125]. The resistance of cancer cells to acidic environments is accompanied by increased activity of extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation and in the presence of PPIs, it has been found that ERK1/2 phosphorylation was inhibited. It is believed that this is involved in PPIs' pro-apoptotic activity [123].

A change in gene expression in rat gastric corpus mucosa *in vivo* was observed in the presence of omeprazole. More importantly, the altered genes encoded for processes such as apoptosis and other stress responses [126]. Omeprazole has been found to induce apoptosis in Jurkat cells (T-cell cell line) in a time- and concentration-dependent fashion. Omeprazole-induced apoptosis is mediated by caspase-3, cysteine cathepsin, and lysosomal enzyme

activity and causes immediate lysosomal destabilization [123,127]. Omeprazole and its acidified/active form (achiral sulphenamide) also induce caspase-dependent apoptosis in polymorphonuclear neutrophils *in vitro* [128].

Effects on non-parietal cells that are due to molecular targets other than H^+,K^+-ATPase inhibition

Effects on contractile systems

Certain cell and tissue effects reported in the literature could be related to H^+,K^+-ATPase inhibition, but are equally likely to point to PPI actions on target proteins other than H^+,K^+-ATPase. An obvious example mentioned previously is the PPI inhibition of cytochrome P450s. Another example, and a source of additional potential side effects, is the action that certain PPIs have on contractile systems. Omeprazole and lansoprazole have been observed to induce relaxation of isolated human arteries, and this relaxation occurred even in K^+-free medium, indicating a probable lack of involvement of H^+,K^+-ATPase. Involvement of intracellular Ca^{++} regulation is implicated [129]. Similarly, an inhibition of contractile activity of (isolated) guinea pig gallbladder smooth muscle strips followed by relaxation was produced by omeprazole and lansoprazole [130]. Isolated rat vas deferens contractility was likewise inhibited by lansoprazole and this effect was also K^+-independent, again suggesting that H^+,K^+-ATPase may not be the target protein [131]. Lansoprazole appeared to inhibit Ca^{++} entry into the muscle through voltage-gated channels. A partially reversible, dose-dependent, negative ionotropic effect on isolated human and rabbit myocardium was demonstrated to occur upon exposure to pantoprazole. This decrease in contractility was thought to be due to depression of Ca^{++} signaling and myofilament activity, and occurred at pH 7.3–7.4, suggesting it is an effect of the pro-drug, not the activated form. Similar results were obtained with esomeprazole suggesting a class effect [132].

Effects on contractile systems regulating cell motility

Two other instances of omeprazole effects on calcium-regulated contractile systems are the inhibition of leukocyte transmigration through endothelial cell sheets [133] and the inhibition of Ca^{++} current in isolated myocytes following administration of omeprazole [134]. Omeprazole and pantoprazole also inhibited migration of human neutrophils, an action that correlated with a decrease in intracellular calcium and decreased activity of p38 MAP kinase, although all of these effects could be downstream of inhibition of a neutrophil H^+,K^+-ATPase since effects could be reversed by a K^+ ionophore [135]. Another effect that may be dependent on MAP kinases is enhancement of gastric epithelial cell proliferation and migration following administration of lansoprazole, an effect suggested to be dependent upon p44/p42 MAPK [136].

Actions on metabolic enzymes

In addition to the cytochrome P450 effects discussed below, omeprazole has also been shown to directly inhibit certain metabolic enzymes such as pyruvate decarboxylase and carbonic anhydrase [112,137]. Red blood cell glucose-6-phosphate dehydrogenase was also inhibited by omeprazole at physiological concentrations [138].

Effects on cell proliferation

In hepatocytes cultures, omeprazole has been shown to possibly increase the rate of DNA repair synthesis, but definitely increasing the frequency of aberrant crypt foci in colons of rats simultaneously treated with azoxymethane [139]. Omeprazole has also been shown to upregulate expression of Insulin-Like Growth Factor (IGF) Binding Protein, thus potentially allowing it to affect IGF-dependent processes [140].

Effects on membrane transport proteins and channels

Another mechanism that omeprazole and other PPIs can use to achieve non-H^+,K^+-ATPase -mediated effects, is through competition with other molecules for their uptake into cells. Omeprazole, lansoprazole, and pantoprazole all inhibited digoxin transport in CACO-2 and L-MDR1 cells, for example, presumably via the P-glycoprotein transporter [141]. Note that this action, like the inhibition of cytochrome P450 isoforms by PPIs, would also act to increase blood levels of certain drugs (like digoxin) that compete for uptake by P450. Unrelated, but noteworthy, omeprazole and lansoprazole also inhibit swelling-dependent chloride channels in fibroblasts [142].

Inhibition of liver cytochromes

In any consideration of PPI actions that are not mediated by H^+,K^+-ATPase inhibition, the major source of such actions is the effect of PPIs on liver cytochrome P450s. These effects have been nicely summarized by Andersson [143]. Omeprazole and other PPIs both inhibit as well as induce particular liver cytochrome P450 isoforms. Omeprazole is metabolized into various metabolites by liver cytochromes in the P450 family. Omeprazole is broken down by CYP2C19 (*S*-mephenytoin hydroxylase) into 5′-hydroxyomeprazole and by CYP3A into omeprazole sulfone, which is further broken down by CYP2C19 into 5′-hydroxyomeprazole sulfone [144,145]. Omperazole, thus, can not only compete as a substrate for the above cytochromes but it can also inhibit the hydroxylation of the CYP2C (specifically 2C19) subfamily [146]. This CYP subfamily is responsible for the metabolism of a number of drugs, including omeprazole, and also diazepam, S-warfarin, and tolbutamide, just to name a few. Considering this, it is hypothesized that inhibition by omeprazole is really due to competition for the binding site of CYP2C. The severity of inhibition/interaction varies depending on the other drugs' properties: whether their metabolism involves cytochromes in addition to CYP2C; their plasma concentration [147]; their metabolic rate [146]; and also the genetics/ethnicity of the person concomitantly taking omeprazole with any other drug(s) metabolized by CYP2C, that is, whether they are a poor or extensive metabolizer of the drug in question as well as of the PPI [144]. It has been found for example that people of white European heritage are at a higher risk for PPI-cytochrome interaction [148].

For omeprazole, there are three types of metabolizers—poor, intermediate, and extensive. Poor metabolizers make up about 3–4% of the Caucasian population and a much higher proportion of the Asian population (20%). Poor metabolizers are known to have higher plasma concentrations and lengthened elimination periods of omeprazole and its metabolites than their intermediate and extensive metabolizer counterparts [148].

It is interesting to note that in addition to CYP2C19, lansoprazole inhibits about four more cytochrome P450 forms than omeprazole does [149]. In rat liver microsomes, pantoprazole had the least inhibitory effect on cytochrome P450s in comparison to omeprazole and lansoprazole, with lansoprazole showing the greatest inhibition [150]. More recent evidence in human liver microsomes suggests that pantoprazole and lansoprazole have the greatest inhibitory effects, but each inhibits a different CYP2C form. Omeprazole has a greater inhibitory profile than esomeprazole, while rabeprazole has the least of all. A metabolite of rabeprazole, however, may exhibit medically relevant inhibitory effects of P450 enzymes [151]. It has also been documented that one of omeprazole's metabolites, omeprazole sulphone, has inhibitory effects regarding diazepam metabolism as well [152]. There is evidence to suggest that repeated use of omeprazole results in an increase in liver weight and cytochrome P450 activity in rats [153].

Turning to PPIs *inducing* cytochromes, it has been confirmed in a human hepatoma cell line that omeprazole induces the synthesis of CYP1A1 (found in hepatocytes) and CYP1A2 (found in enterocytes). There are many inducers of CYP1A in addition to omeprazole, and, in fact, healthy dietary agents like Brussels sprouts and broccoli, which are thought to reduce the incidence of cancer, are more potent inducers than omeprazole. Cigarette smoke, physical exercise, and charcoaled beef are also considered inducers of CYP1A. It has been suggested that effects of omeprazole on CYP1A are negligible in comparison to dietary effects on CYP1A. The mechanism of induction involves the Ah (aromatic hydrocarbon) liganded receptor, which is bound to the inducer, followed by translocation into the nucleus, and then signaling for CYP1A gene transcription [147]. There is evidence, however, that omeprazole is unable to competitively displace 2,3,7,8-tetrachlorodibenzo-p-dioxin (a typical Ah ligand) from the Ah receptor, suggesting its induction effects may be insignificant [147,154]. In fact, in a cell culture model, the induction of CYP1A1 by omeprazole and lansoprazole did not involve the Ah receptor, that is, their induction is ligand-independent.

It has been demonstrated *in vivo* that omeprazole, at therapeutic doses (20 mg/day), induces CYP1A1 mRNA, and evidence of CYP1A1 was found to be present in different areas of the gastrointestinal tract, including the presence of CYP1A2 in the duodenum [155]. Other investigators, however, state that omeprazole (20 mg/day) and lansoprazole (30 mg/day) do not induce CYP1A2 *in vivo*, except in poor metabolizers [156,157]. In isolated rat hepatocytes, omeprazole was found to induce CYP1A1 in a dose- and time-dependent manner [154] In isolated human hepatocytes, CYP1A2 was induced by omeprazole in a time- and concentration-dependent manner [158].

The clinical significance of these actions of PPIs on cytochromes, especially inhibition, is that they can affect the clearance of certain drugs from the bloodstream, and thereby affect the plasma concentration of these drugs. This may lead to the types of serious side effect/interaction seen by Kiley *et al.* [159] for omeprazole and digoxin. Rabeprazole, which, like pantoprazole, is considered to have fewer such drug interaction effects than omeprazole, has been observed to increase the maximal plasma concentration of digoxin by almost 30% [160].

Effects on bone tissue

Aside from the actions cited above on contractile systems, another example of omeprazole-related effects on Ca^{++} regulation is in bone. Omeprazole inhibits prostaglandin and parathyroid hormone-stimulated Ca^{++} release from neonatal calvariae [161]. Whereas the major proton ATPase of the osteoclast is the vacuolar type, which has many differences from the H^+,K^+-ATPase and is relatively unaffected by PPIs, omeprazole did decrease Ca^{++} excretion in urine, also suggesting an effect of PPIs on bone resorption [162].

Miscellaneous clinical effects attributed to PPIs

PPIs have also been linked to a variety of adverse reactions seen in the clinical setting. One group of investigators in Auckland, New Zealand have cited PPIs as the most commonly identified cause of acute interstitial nephritis in their area with an incidence of 1:12 500 patient years on a PPI. Though the etiology of this reaction has not been identified, it may be a result of direct toxicity of the drug – or one of its metabolites – causing an interstitial inflammatory response [163]. PPIs have also been thought to induce inappropriate secretion of antidiuretic hormone resulting in hyponatremia that resolved after discontinuation of the drug [164]. Though this is a rare occurrence and definite causation by PPIs is difficult to prove, it should be considered in the evaluation of a patient with hyponatremia. Likewise, patients with myopathies, including polymyositis, should be questioned about their use of PPIs as there is evidence of a causal relationship. PPI induced auto-antibodies are the proposed mechanism of this reaction [108].

Recent case reports have linked *long*-term use of PPIs with severe hypomagnesemia, in two cases resulting in hypocalcemic seizures [165,170]. Low levels of magnesium in the patients' urine indicate there was no inappropriate renal loss. It has been proposed that PPIs induce a defect in active magnesium absorption in the small intestine. Whether this is due to an action on the Mg^{++}-ATPase or on the tight junctional proteins is not yet known. The hypomagnesemia may be connected with the elevated luminal pH produced by PPIs, but even that is still unproven [165]. The cases reported involved patients who had been on PPIs for at least one year. There is no evidence that *short*-term PPI use alters intestinal absorption of magnesium [171]. In all reported cases electrolyte abnormalities corrected with withdrawal of the PPI.

Having given this litany of PPI effects that may not be directly attributable to H^+,K^+-ATPase inhibition – a list of actions that seem alarming in their broadness – it is necessary to balance these findings with the equally important realization that omeprazole has been shown to be negative for short-term mutagenicity tests, the Ames test, the micronucleus test in mice, and the mouse lymphoma test [53].

Induction of transepithelial gastric leak by PPIs

The following newly discovered side effect of PPIs is a very good example of the unexpected nature of side effects, their frequent apparent discontinuity from their intended action, and sometimes even their counterintuitive nature. It merits its own section because: (1) it affects both gastric and non-gastric epithelia; (2) cannot as yet be definitively linked to H^+,K^+-ATPase inhibition or to a non-H^+,K^+-ATPase target; and (3) may derive more from

inhibition of acid secretion in general than to specific, direct inhibition of the H^+,K^+-ATPase. It is still unknown whether this particular side effect has clinical ramifications, but for the above-mentioned characteristics, it serves as a fitting close to the review.

PPIs, by virtue of reducing gastric acid secretion, are well known for allowing morphological healing of damage to gastric and esophageal mucosa resulting from conditions such as gastritis or reflux disease (GERD). Because of this property, one might assume that PPI use would allow for improvement in epithelial barrier function of the upper GI tract in patients with such acid-related disease. Our group, however, observed exactly the opposite effect on barrier function, in what represents a classic example of an unexpected medication side effect. When transepithelial permeability of the upper gastrointestinal tract was measured in first-time-presenting reflux disease patients (who were either PPI naïve or at least off PPI [or H-2 blocker H2RA] medications for 30 days) and then measured again after an eight-week course of esomeprazole therapy (which alleviated symptoms in 100% of patients enrolled), a surprising 300% increase in transepithelial permeability (leak) was observed [166]. This leak set in as soon as blood levels of the medication plateaued (three to five days of a 40 mg/day dose), and reversed within days of stopping the medication.

An omeprazole-induced leak had, in fact, been reported earlier for rat gastric corpus, an effect dependent upon prior stimulation of acid secretion with secretagogues [167]. We have observed – also using rat gastric corpus – the following characteristics of this omeprazole-induced leak: (1) it manifests a similar dose-dependence to that observed for inhibition of acid secretion with a half maximal effect at approximately 10 μM; (2) it appears immediately upon exposure of the tissue to esomeprazole; (3) it allows for transepithelial permeation of uncharged solutes as large as 4 kDa, but not solutes 10 kDa or greater; (4) it is bidirectional; (5) it is an apparent class effect, with omeprazole, esomeprazole, and lansoprazole producing similar effects [168], and it ensues whether acid suppression is achieved by histamine blockers or by proton pump inhibitors (unpublished data).

The fact that there is a size limit to solutes able to pass through this leak suggests that the mechanism is not due to PPIs inducing cell death in the gastric corpus, with subsequent cell detachment allowing for leak. The bidirectional nature of the leak and its permeation by solutes such as mannitol (unable to enter cells) suggests that an alteration of tight junction permeability may have occurred. It is a surprising result in that an inhibitory effect on the parietal H^+,K^+-ATPase would not necessarily be expected to translate itself into a tight junctional barrier change. We have, however, referenced above, studies where PPIs have affected contractile/cytoskeletal systems and calcium homeostasis [129,130], so in hindsight an effect on the tight junction (an 'extension' of the cytoskeletal system of the cell) is not outside the realm of possibility.

The implications of this leak – still to be explored – could be threefold. First, if the leak allows for permeation across the gastric barrier of certain oral medications that a patient is taking while on a PPI, it is theoretically possible that this could result in unintended elevation of blood levels of such medications. Given the fact that PPIs also inhibit the CYP2C subclass of cytochrome P450s and thereby slow down the rate of clearance of certain drugs from the bloodstream, one could have two effects working in concert to elevate blood levels and potentially create dangerous side effects of such drugs, as described in Kiley *et al.* for digoxin [159].

The second scenario to consider is the penetration across the gastric barrier of naturally occurring substances, such as food antigens, and the effect that may have on allergic/inflammatory disease. In this same vein, there are peptides and small proteins in salivary secretions (which continually bathe the gastric mucosa) such as Epidermal Growth Factor (EGF) (which exists in saliva at concentrations as much as 1000× the concentration in the bloodstream), which if able to permeate across the PPI-induced leak, could enter the gastric stroma and lead to a spectrum of cell kinetic and angiogenic changes in the gastric mucosal stroma and epithelium [169]. The gastric wall thickening, cell hyperplasia, and fundic polyposis that are documented to occur frequently with PPI use have been described above and are universally attributed to PPI-induced hypergastrinemia. EGF penetration into the stroma from mucosal salivary secretions could, however, be contributory to these events, and gastrin may not be the entire cause.

The third potential scenario (assuming the above two negative scenarios are found not to be significant) would be whether a PPI-induced gastric mucosal leak could actually be utilized to allow for oral delivery (and gastric uptake) of medications that must normally be given intravenously. These could be peptides, small proteins, or oligonucleotides that may not have appreciable uptake through the small bowel and may also be degraded once entering the duodenal lumen. If these substances pass through the PPI-induced leak at a consistent, reproducible rate (picomoles/unit time/unit gastric surface area) that is appreciable enough to deliver a pharmacologically meaningful dose into the bloodstream, conceivably another use for PPI medications may present itself.

Summary

No drug, however well targeted, can be without side effects. Even if a drug is targeted to one specific receptor, one specific kinase, or one specific phosphatase, what we now know about the domino chain-like interconnections and intersections of intracellular signaling pathways means that effects other than the intended, desired effect will, in fact, ensue. In addition, any drug administered systemically may be impacting its molecular target, not only in tissue *x* where its effect is desired, but in tissues *y* and *z*, where the kinase, phosphatase, or receptor also exists, and an effect may neither be desired nor expected. For example, a drug that inhibits protein kinase C epsilon (and only protein kinase C epsilon) may achieve its desired effect in liver, but produce unwanted effects in lung and kidney, where protein kinase C epsilon may be positioned in quite different intracellular signaling pathways. As targeting efforts in drug design become more developed and advanced, the burden should still be to test for and expect the 'unexpected' and undesired action. That testing should be very inductive, that is, an attitude of 'let us see what it does' rather than heavily rational testing that assumes beforehand what it will/will not do. Who would have predicted that H^+,K^+-ATPase inhibition would induce transepithelial leak? The very byzantine quality of intracellular signal transduction pathways means that the 'testing' of a drug is never really finished as long as it is being utilized.

As with certain other drugs, the potentially more serious side effects of PPI use may well be highly idiosyncratic. This is certainly true in the inhibitory actions of PPIs on the cytochrome P450s and the effects these will have on clearance of other medications from the bloodstream. The abovementioned, newly described phenomenon of PPI-induced gastric leak may contribute as well. Genetic polymorphisms may exert a key role in both side effects. So too will the type of PPI being prescribed, as well as the nature of the administered drug under consideration (e.g. digoxin, warfarin, phenytoin, and so on) and its affinity for the cytochrome in question. We would expect similar idiosyncrasy in potential effects of PPIs on non-parietal H^+,K^+-ATPases as well as possible non-H^+,K^+-ATPase mediated effects. For the history so far in hand, PPIs are generally safe and well tolerated. There are, however, cases of highly serious, PPI-driven side effects as described in Kiley *et al.* [159]. In addition, it is possible that the generally regarded benign morphological side effects of PPIs may not be so benign when tested against a population at very high risk of gastric cancer (as in northwestern China). Physicians need to be very aware that PPIs are interacting with multiple molecular targets, and assume that any patient may be an idiosyncratic issue with regard to unintended effects.

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