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Barrett's esophagus: proton pump inhibitors and chemoprevention I

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The following on proton pump inhibitors and chemoprevention in Barrett's esophagus includes commentaries on normalization of esophageal refluxate; the effects of 5-HT_4 agonists on EGF secretion and of lubripristone on chloride channels agents; the role of Campylobacter toxin production; the deleterious effects of unconjugated bile acids; the role of baclofen in nonacid reflux; the threshold for adequate esophageal acid exposure; the effects of proton pump inhibitor (PPI) therapy on normalization of esophageal pH and on cell proliferation; the role of the phenotype of cellular proliferation on the effects of PPI therapy; and the value of Symptom Index and Symptom Association Probability in the evaluation of potential response to treatment.

Keywords: Barrett's treatment; omeprazole; duodenogastroesophageal reflux; dysplasia; Symptom Sensitivity Index; Symptom Association Probability; antireflux surgery; PPI; mucin phenotype; pH control; esomeprazole; basal cell layer; hyperplasia; stromal papillae; $GABA_B$ baclofen; lesogaberan; weakly acidic reflux; TLESR; Campylobacter; microbiota; prostone; 5-HT_4; TGF-α; GERD; EGF; mucosal protection; CDX2; Barrett's cell lines

Concise summaries

- *In vitro* data in cultured cells suggest that acid and bile exposure is important in Barrett's carcinogenesis. Normalization of esophageal acid exposure—albeit not formally proven in RCT studies—should be beneficial in preventing metaplasia in gastroesophageal reflux disease (GERD) patients and potentially diminish the likelihood of neoplastic progression.
- Esophageal preepithelial mucosal defense mechanisms in patients with BE are significantly impaired, potentially predisposing metaplastic epithelium to further injury, chronic inflammation and progression to esophageal adenocarcinoma.

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- 5-HT_4 receptor expression significantly increases from the controls to GERD and to BE patients. 5-HT_4 could play a role, involving both EGF and COX-2 pathways, determining PGE2 secretion increase and, consequently, proliferative activity increase, and 5-HT_4 selective antagonists could be the future of GERD therapy and BE chemoprevention.
- The impact of chloride channel stimulators on secretion of protective factors in disease of the esophageal mucosa, especially in patients with Barrett's esophagus (BE) with and without low/high-grade dysplasia (LGD/HGD), remains to be explored.
- Acid suppression therapy with proton pump inhibitors (PPIs) may lead to the bacterial overgrowth and increased reflux of toxic, unconjugated bile acids. This may result in increased cell DNA damage, mutations, and consequently to BE and EAC development. However, the current knowledge about esophageal colonization with Campylobacter species does not justify eradication with antibiotics.
- Most groups currently define adequate acid control in GERD patients as values below normal thresholds for healthy controls, usually a total acid exposure time of less than 4.5–5.5%.
- In patients in whom ongoing symptoms are related to nonacidic reflux events, the reduction of TLESR frequency $GABA_B$-receptor agonists is a potentially beneficial therapeutic strategy. Baclofen is a reasonable therapeutic add-on in patients under PPIs with persistent symptoms due to nonacid reflux.
- The epithelium of patients with continuous acid exposure is believed to only partially benefit from PPI therapy, whereas the epithelium of patients with pulse acid exposure, leading to more pronounced epithelial changes, might have a greater potential to be positively influenced/cured by PPI therapy.
- For an antisecretory treatment aimed at chemoprevention to be effective, higher PPI dosing, confirmed by pH monitoring, is necessary. PPIs suppress cellular proliferation in BE with the gastric-predominant mucin phenotype, but not in that with the intestinal-predominant mucin phenotype. This finding may partly explain the ongoing controversy effects of acid-suppressive therapy in Barrett's patients.
- Great caution is recommended when using Symptom Sensitivity Index and Symptom Association Probability results to predict a response to PPIs in individual patients, especially in those without classic heartburn or regurgitation.
- There is no significant association between esophageal adenocarcinoma and the use of antisecretory agents *per se*.

1. Should the esophageal refluxate be normalized in BE? *In vitro* studies to answer this question

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Two retrospective studies have suggested that controlling esophageal pH may decrease the chances to develop dysplasia. In a VA study, El Serag *et al.*[1] compared the development of dysplasia in patients with BE treated with or without PPI or histamine 2-receptor antagonist (H2RA) over a 20-year time period. They found that the cumulative incidence of dysplasia was significantly lower among patients who received PPI after BE diagnosis than in those who received no therapy or H2RA. Furthermore, among those on PPIs, a longer duration of use was associated with less frequent occurrence of dysplasia. In the second study,[2] Hillman *et al.* examined whether PPI therapy influences the incidence and progression of dysplasia in patients with BE. They found that ongoing PPI therapy appeared beneficial in the prevention of dysplasia and adenocarcinoma in patients with BE and suggested that all patients with this condition, even those with no esophagitis or symptoms, should be encouraged to continue long term PPI therapy.

Hence, control of the esophageal acid (and bile) exposure by mechanical and pharmacologic means

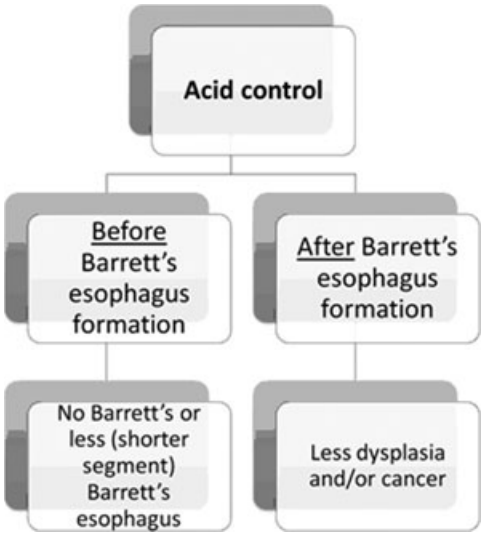

Figure 1. **Outline of two scenarios that relate acid control and Barrett's pathogenesis and natural history. Originally published in Ref. 3.**

seems quite important in the pathogenesis and natural history of BE. Figure 1 highlights two possible scenarios:[3]

(i) Under the first scenario, acid control before the development of BE may either abort the formation of metaplasia or be associated with shorter segment metaplasia.
(ii) Under the second scenario that occurs after the formation of metaplasia, effective acid control could lead to less dysplasia and cancer, a chemoprevention effect.

Several recent *in vitro* studies have explored the role of the refluxate, such as acid and bile, in affecting BE formation and inducing alterations in the Barrett's cells that would, in turn, favor malignant transformation, but, for brevity, only two recent ones are highlighted herein.

Under the first scenario, Huo *et al.* hypothesized that differences among individuals in molecular pathways activated when esophageal squamous epithelium is exposed to reflux underlie the development of Barrett's metaplasia.[4] They used esophageal squamous cell lines from patients who had GERD with BE and without BE to study the effects of acid and bile salts on expression of the CDX2 gene. They found that both acid and bile salts increased CDX2 messenger RNA (mRNA), protein, and promoter activity in NES-B3T and NES-B10T cells, but not in NES-G2T or NES-G4T cells. They also found CDX2 mRNA in 7 of 10 esophageal squamous biopsy specimens from patients with BE, but in only 1 of 10 such specimens from patients who had GERD without BE. Since acid and bile salts induce CDX2 mRNA and protein expression in esophageal squamous cells from patients with BE, but not from GERD patients without BE, they speculated that these differences in acid- and bile salt-induced activation of molecular pathways may underlie the development of Barrett's metaplasia.

Hong *et al.* examined whether acid increases methylation of p16 gene promoter and whether NADPH oxidase NOX5-S mediates acid-induced p16 hypermethylation in a Barrett's cell line BAR-T and an EA cell line OE33.[5] Inactivation of the tumor suppressor gene p16 may be important in the malignant transformation of BE. Hypermethylation of p16 gene promoter is an important mechanism inactivating p16. They found that NOX5-S was present in BAR-T and OE33 cells and that acid-induced increase in H_2O_2 production and cell proliferation was significantly reduced by knockdown of NOX5-S (Fig. 2). Exogenous H_2O_2 remarkably increased p16 promoter methylation and cell proliferation. In addition, acid treatment significantly increased p16 promoter methylation and decreased p16 mRNA level. Knockdown of NOX5-S significantly increased p16 mRNA, inhibited acid-induced downregulation of p16 mRNA, and blocked acid-induced increase in p16 methylation and cell proliferation. Conversely, overexpression of NOX5-S significantly decreased p16 mRNA and increased p16 methylation and cell proliferation. The authors postulated that acid reflux may activate NOX5-S and increase production of reactive oxygen species, which, in turn, increase p16 promoter methylation, downregulate p16

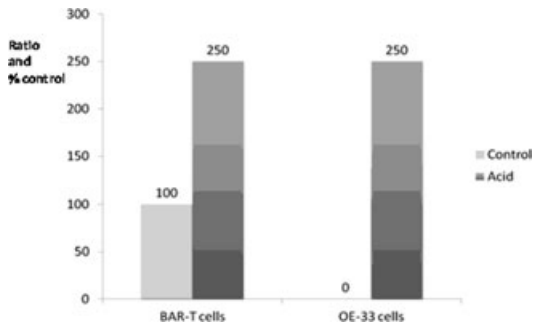

Figure 2. **Acid increases methylation levels of p16 gene promoter in BAR-T cells and OE33 cells *in vitro*.**

expression, and increase cell proliferation, thereby contributing to malignant progression.

In conclusion, recent *in vitro* data in cultured cells suggest that acid (and bile) exposure is important in Barrett's carcinogenesis. Normalization of esophageal acid exposure—albeit not formally proven in RCT studies—should be beneficial in preventing metaplasia in GERD patients and potentially diminish the likelihood of neoplastic progression of BE.

2. Are esophageal mucosal defense mechanisms different in BE?

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GERD is defined as the presence of chronic symptoms, predominantly including heartburn/regurgitation with or without mucosal damage produced by an abnormal reflux, i.e., retrograde flow of gastric or mixed duodeno-gastric content into the esophagus and/or mouth. Considering the fact that gastroesophageal refluxate (GER) contains a range of injurious agents, from acid accompanied by seven to eight pepsins and to bile acids mixed with acid/pepsins and pancreatic enzymes, esophageal mucosa has to have in place a plethora of protective factors counteracting these aggressive components, thus preventing injury to the squamous epithelium. Among the esophageal preepithelial protective factors elaborated by salivary or esophageal submucosal mucous glands, buffers and mucins are the leaders, followed by epidermal growth factor (EGF) and transforming growth factor-α (TGF-α), representing a vanguard of mucosal protection in health and disease.[6]

The concept of the role of mucosal protection in health and disease

If protective mechanisms are inadequate or overwhelmed qualitatively or quantitatively by an excessive injurious refluxate, mucosal injury and subsequent repair will take place, thus setting the stage for acute/chronic inflammation.[7,8] It is squamous epithelial repair, in an environment of unabated reflux and dropping pH within the mucosa, that leads highly proliferating mucosal stem cells to replace injured cells, to turn on mucosal differentiation into columnar epithelium, called BE, which is better equipped to cope with the luminal content of gastroduodenal injurious components.[7] Within 24 h, the composition of GER varies profoundly, from highly and predominantly acidic, especially during the after midnight hours, to highly contaminated with duodeno-gastric reflux during, and shortly after, ingestion of various meals at day time. This continuous variation in the composition of injurious components within GER sets the stage for the final phenotype of Barrett's mucosal cells as incomplete intestinal metaplasia (the mixture of gastric and intestinal cells with some even expressing goblet cell morphology) that is still a subject of injury/repair, as this columnar epithelium exhibits still inadequate mucosal protection.[6–8] This is why highly injurious and proinflammatory stimuli maintain chronic inflammation resulting, in some patients, in the development of complications such as LGD, HGD, and ultimately esophageal adenocarcinoma (AdCa).[6–8]

Although pathophysiology of GERD/BE includes pan-esophageal motility disorder, including defective lower esophageal sphincter (LES), excessive transient LES relaxation (TLESR), and impaired primary and/or secondary esophageal peristalsis and clearance, it is the degree of imbalance between aggressive factors and protective mechanisms that will define absence or presence its final outcome, esophageal adenocarcinoma.[6–8] Setting the stage for potential development of BE, patients with reflux esophagitis have significant impairment in esophageal mucin secretion, as well as esophageal and salivary EGF, which persist even after healing of erosive changes, indicating that this preexisting condition facilitates the development of mucosal injury.[6] Furthermore, esophageal EGF secretion also remains impaired in patients with BE.[9]

Although therapy with PPIs improves the equilibrium between aggressive factors and protective mechanisms by diminishing acidity of GER, some PPIs, such as rabeprazole, may also increase esophageal secretion of protective mucin after healing of erosive mucosal changes.[10] Administration of the serotonin receptor (5-HT_4) agonist tegaserod, and potentially other newer agents free of cardiac side effects, results in significant increase in salivary bicarbonate and nonbicarbonate buffers, EGF, and TGF-α, as well as esophageal EGF,[6] promoting further potential restoration of equilibrium. This enhancement of equilibrium can be also promoted further by mastication or chewing sugarless

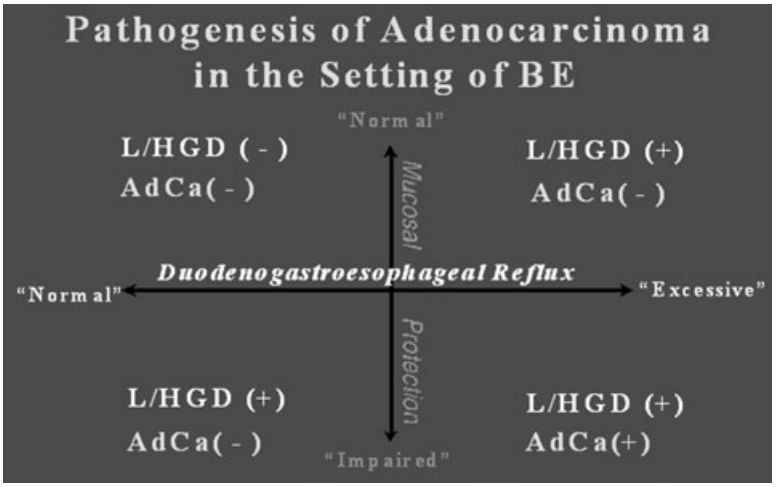

Figure 3. **The relationship between disequilibrium between aggressive factors and protective mechanisms in the development of L/HGD and esophageal adenocarcinoma in patients with BE.**

chewing gum, which leads to a two- to four-fold increase in salivary buffers, mucin, EGF, and TGF-α.[8] Obviously, administration of $GABA_B$ receptor agonists (baclofen and the newest agents tested in clinical trials) help to prevent GER through inhibition of TLES relaxation.[7] Perhaps in some patients with BE who still exhibit significant GER in spite of BID doses of PPIs, addition of sugarless chewing gum- and/or $GABA_B$ receptor antagonists or 5-HT_4 receptor agonist could lead to restoration of equilibrium, thus diminishing profoundly subsequent complications such as LGD, HGD, and adenocarcinoma. This, however, requires confirmation in randomized, placebo-controlled, double-blind clinical trials.

Theoretically, fundoplication, if perfectly performed, should have the greatest potential of inhibition of not only reflux of the acid/pepsins component of GER but also its duodeno-gastric mixture.[7] Its adenocarcinoma preventive potential in patients with BE still remains to be demonstrated. Our illustration (Fig. 3) may help to outline the role of equilibrium between aggressive factors within duodeno-GER and protective factors within salivary and esophageal secretions in maintaining the integrity within the BE mucosa and prevention of the development of esophageal AdCa.

If pharmacological or surgical therapy normalizes the quality and the quantity of duodeno-gastroesophageal reflux and mucosal protective factors remain normal, LGD/HGD should not develop (left upper panel); however, if mucosal protection still remains low (left lower panel), L/HGD may develop but could remain nonprogressive. If pharmacological or surgical therapy diminishes but does not normalize the quality and the quantity of DGER and mucosal protection remains strong or normal, L/GHD could still develop without progression to AdCa (right upper panel); however, if mucosal protection also remains impaired (right lower panel), L/HGD may inevitably develop and exhibit progression to AdCa.

Conclusion

Esophageal preepithelial mucosal defense mechanisms in patients with BE are significantly impaired, potentially predisposing metaplastic epithelium to further injury, chronic inflammation, and progression, through LGD and HGD, to esophageal adenocarcinoma.

3. What is the effect of 5-HT_4 agonists on esophageal EGF secretion in patients with GERD and BE?

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Serotonin (5-HT) was discovered in intestinal cells by an Italian researcher, Vittorio Erspamer, in the early 30s. Since then both agonists and antagonists drugs towards 5-HT receptors have been

particularly used for gastrointestinal motility disorders, such as constipation, irritable bowel syndrome, nausea, and vomiting.[11,12] There are many 5-HT receptors subtypes and some of them play a mitogenic role throughout their activation.[13] So far, there is no evidence concerning the role of 5-HT_4 receptors in GERD and BE patients. It is well known that EGF is very important in the regulation of the tissue integrity and its receptors seem to play a role in cell proliferation as well as in oncogenesis of the gastrointestinal tract.[14] Moreover, these receptors are present throughout all the intestine and are significantly increased in GERD.[15]

Preliminary experience

This preliminary study includes 22 patients, divided in to three groups: controls, GERD, and BE patients. Esophageal samples were taken from each patient and cells were isolated and studied in order to evaluate cell proliferation, Western blotting, and secretion analysis.

5-HT_4 receptor expression, although present in all the patients, significantly increases from controls to GERD patients and then to BE patients.

Our preliminary data show that proliferation, evaluated by means of tritiated thymidine incorporation, significantly increases from basal levels in each of the three studied groups after incubation with 5-HT. This effect is more evident after cell preincubation with 5-HT agonists, such as Cisapride, a partial agonist for both 5-HT_3 and 5-HT_4 receptors, and CJ033466, which is a selective 5-HT_4 receptor agonist. On the contrary, preincubation with a selective HT_4 antagonist reverses proliferative activity to basal levels.

EGF expression and secretion was also studied, and our findings show that EGF expression increases from controls to BE patients. These data are confirmed by the analysis of the secretion in basal conditions in the three groups. Incubation with Cisapride or selective HT_4 agonists significantly increases EGF secretion, and the selective antagonist reduces it to basal levels (Fig. 4). In addition to 5-HT_4 receptors, EGF has the same effect on proliferative activity evaluated by means of thymidine incorporation in the three groups. Preincubation with EGF significantly increases proliferative activity in the three groups, on the contrary, preincubation with a selective COX-2 inhibitor, such as Celecoxib, reduces proliferative activity to basal level. Finally, our data show that 5-HT agonists and, mostly, selective HT_4 agonists increase PGE2 secretion. Conversely PGE2 is decreased by the selective 5-HT_4 antagonist that, therefore, determines the COX-2 activity increase.

In conclusion, in GERD and BE patients, 5-HT_4 could play a role that involves both EGF and COX-2 pathways, both determining PGE2 secretion increase, and consequently proliferative activity increase. Our preliminary data suggest that the 5-HT_4 receptor involvement may be very important, at an early stage of GERD multistep process, inducing cell proliferation by means of EGF and/or COX-2 increased activity. Therefore, 5-HT_4 selective antagonists could be the future of GERD therapy and

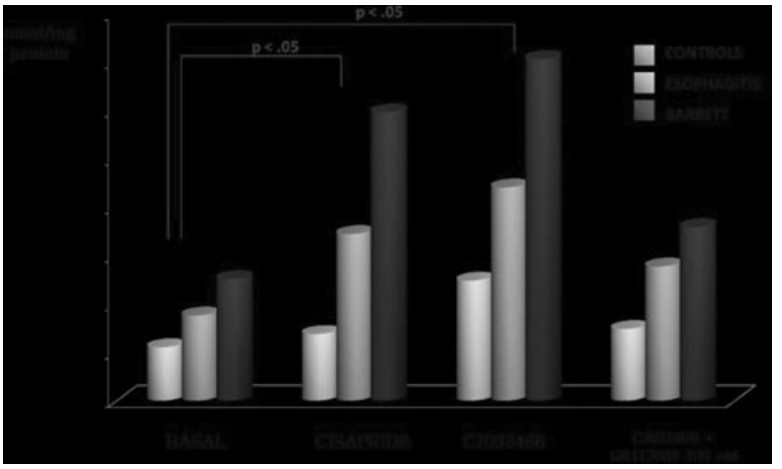

Figure 4. **Incubation with cisapride or selective HT_4 agonists significantly increases EGF secretion, and the selective antagonist reduces it to basal levels.**

BE chemoprevention, but further both experimental and clinical studies in order to support this hypothesis are needed.

4. What is the effect of lubiprostone on chloride channel-2 (CIC-2) driven secretion of protective components of mucus in the human esophagus?

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The alimentary tract mucosa is covered by a viscoelastic, approximately 200 micron thick, mucus–buffer layer. The primary role for this layer is to protect the delicate surface epithelium from various chemical and physical aggressive factors and forces, either elaborated endogenously or from exogenous food components, providing also lubrication facilitating propagation and propulsion of nondigestible food solids. There is a close interrelationship between the rate of mucus release from the gastrointestinal mucous cells or from submucosal mucous glands within the esophageal mucosa, and the rate of chloride secretion, setting the optimal conditions for hydration of mucus and formation of its viscoelastic gel layer. Chloride channels type 2 (CIC-2) are widely distributed in tissues through the body and are expressed in many epithelia, especially within the alimentary tract. Lubiprostone, a member of a group of compounds called prostones, within the family of prostanoids, is a locally acting chloride CIC-2 channel activator that enhances a chloride-rich fluid secretion, subsequently resulting in an increase of the alimentary tract secretion, including mucus, driven by stimulation of chloride sodium, thus accelerating transit and alleviating symptoms associated with chronic constipation regardless its etiology. It has been clearly demonstrated that the human esophageal mucosal secretory response is closely related to its luminal exposure to acid and pepsin, thus setting the stage for its role in esophageal mucosal protection during gastroesophageal reflux. This secretory response is augmented by serotonin receptor 5-HT_4 agonist. Therefore, administration of lubiprostone may result in increase of secretion of other protective factors within the mucus-buffer layer such as prostaglandins, EGF, transforming growth factor α (TGF-α), thus enhancing the esophagel preepithelial defence mechanisms. Hypothetically, by promoting the quantity and the quality of the mucus barrier along the alimentary tract, lubiprostone could also be of value in mucosal protection during administration of nonsteroidal–antiinflammatory drugs (NSAIDs), well known for their ulcerogenic potential both within the upper and the lower alimentary tract, mediated by an impairment of COX-1-generated prostaglandins and subsequent depletion in mucus production, thus increasing the risk of complications.

The integrity of the alimentary tract mucosa depends upon equilibrium between aggressive factors and defense mechanisms.[16] Since aggressive factors within the alimentary tract always act on the luminal side of the mucosa, preepithelial defense, defined as the mucus-buffers layer with its inherent pH gradient, represents the vanguard of mucosal defense.[17] Mucin, also called mucus glycoprotein, generates the architectural framework or scaffold within the mucus layer supporting contribution of other protective components, including hydrophobic phospholipids and buffers to its ultimate defensive potential.[16,17] Therefore, the rate of synthesis and secretion of mucin is pivotal for the thickness of the mucus-buffers layer and its ability to inhibit hydrogen ion back-diffusion and maintain a pH gradient, from acidic on its luminal perimeter to near neutral at mucus–epithelial cell membrane inter phase.[18] Administration of a conventional NSAID, leading to the development of peptic ulcer disease (PUD), or inducing symptoms of nonulcer dyspepsia (NUD) or chronic constipation, results both in decreased generation of gastroprotective prostaglandins generated by COX-1 and diminished production of gastric mucin.[19]

We have recently demonstrated, in a double-blind, placebo-controlled study protocol where administration conventional NSAID, naproxen, 500 mg bid, resulted in a significant decline of gastric mucus production by 44% ($P < 0.001$) in basal conditions and by 35% ($P < 0.001$) in pentagastrin-stimulated conditions, mimicking the food-stimulated scenario.[19] Furthermore, the rate of secretion of gastric mucin, the major component of mucus, during naproxen administration, declined by 39% in basal conditions ($P < 0.01$) and by 49% in pentagastrin-stimulated conditions ($P < 0.005$).[19] Of note, administration of rabeprazole, one of the most effective PPIs, resulted in significant

restorative capacity on naproxen-induced decline of mucus secretion bringing it rate of secretion almost to prenaproxen levels.[19] This decline of mucus and mucin production during administration of naproxen may at least partly explain the propensity of patients receiving NSAIDs for the development of alimentary tract symptoms and complications.

An interrelationship exists in the esophageal mucosa between secretion of mucin and other protective mucus components and chloride through ClC-2 within the alimentary tract.

It is hard to overestimate the value of adequate chloride secretion into the alimentary tract lumen, which is pivotal in regulating optimal rate of hydration of the luminal content, securing adequate fluidity, and promoting its adequate propulsion along the alimentary tract.[20,21] Furthermore, chloride secretion is instrumental in secretion and hydration of gastric mucus and its ability to form gel-like, viscous physical property, instrumental in mucosal protection and lubrication. Recently, a novel agent, the chloride channel activator lubiprostone, has been introduced into our clinical armamentarium, targeting an important pathogenetic link leading to chronic constipation.[20,21] The stimulatory impact of lubiprostone on mucus and mucin production and its viscous, gel-like forming property in asymptomatic volunteers and patients with chronic constipation has recently been explored (unpublished data).

Future implications

The impact of chloride channel stimulators, especially from the family of prostanoids, as well as from the group of prostones on secretion of protective factors such as sodium bicarbonate, mucin, EGF, and TGF-α from salivary and esophageal submucosal mucous glands in the health and disease of the esophageal mucosa, especially in patients with BE with and without LGD/HGD, remains to be explored.

5. In view of the high levels of toxin-producing Campylobacter species found in Barrett's patients, should prophylactic use of antibiotics be considered?

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BE is a premalignant state leading to adenocarcinoma of the esophagus, its close proximity to the stomach raises the obvious question whether bacteria also play a role in disease progression in the esophagus. The GI tract plays host to a large range of bacteria, which helps the physiological process of digestion, while boosting immunity to microbial and other antigens from birth. The immune system requires the presence of commensal bacteria to facilitate its development and maintain its natural immunity to pathogenic microorganisms. The microbiota, and its dysregulation, lead to chronic inflammation, as seen in gastric cancer and possibly colon cancer. In the gastro intestinal tract, these organisms play a major role in immune regulation, and therefore changes in bacterial composition in GERD due to cellular modifications may initiate or maintain neoplastic progression to esophageal carcinoma. So the first step to answering the posed question is what bacteria are present in the esophagus?

The esophagus can be infected with a number of organisms, including *Candida, Cryptococcus*, mycobacteria, and herpes virus. However, studies by Osias *et al.*,[22] Pei *et al.*,[23] and McFarlane *et al.*[24] have demonstrated a biofilm in the esophagus. They have identified many species of bacteria belonging to different genera and phyla. Although the majority of species are of an oral origin, the predominant oral phyla, spirochaetes are not present, providing evidence that not all oral bacteria can colonize these tissues. Studies have been done on the bacterial colonization in patients with reflux esophagitis and BE. Several groups of bacteria have been identified like Streptococci, Staphylococci, Gemella, Veillonella, Neisseria, Prevotella, and Fusobacteria. Although total colony forming units in healthy and BE patients were similar, there is great species diversity.[23–25] More recently, studies have shown the continuation of this colonization of the esophagus by campylobacter into those with esophageal carcinomas. The five bacterial phyla that are commonly identified in all patients at different stages of the reflux–GERD–Barrett's spectrum are: Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, and Fusobacteria. The above studies did not reveal any specific organisms that were present in the majority of disease phenotypes that did not occur in any of the healthy controls. Nevertheless, differences in the prevalence of particular bacterial

groups, and relative bacterial numbers were observed, with the microbiota becoming increasingly Gram-negative. *Campylobacter* manifested the greatest increase during disease progression. The increased presence of potentially pathogenic nitrate-reducing *Campylobacter* species in disease patients is of concern. The increased prevalence of nitrate-reducing *Campylobacter concisus*, not only in BE patients as found previously but also in those with GERD and ADC, may increase the mutagenic potential of refluxate.

This evidence confirms the presence of these bacteria in association with disease progression, and they have biological effects that could enhance mutagenesis or mitogenesis within the esophagus, but, thus far, there is no evidence of causality. This provides justification for further research but little evidence on which to base therapy upon. Turning again to the question posed, we have to ask if antibiotics are the correct therapy to consider, if we did want to eradicate campylobacter from the esophagus. The paradigm proposed is based directly on experience of *Helicobacter pylori* eradication in the stomach, where there is a very selective niche for colonization and an acid barrier to prevent recolonization. The same conditions do not apply in the esophagus where there is a very diverse microbiota and a ready reservoir of bacterial recolonization from the mouth. This would require continuous use of antibiotics with the attendant problems of resistance and secondary infections.

Therefore, the current knowledge about the microbiota of the esophagus, and in particular it's colonization with *Campylobacter* species, does not justify eradication with antibiotics.

6. What is the link between presence of unconjugated bile acids and the deleterious effect of refluxate in patients taking PPIs?

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In BE, normal esophageal mucosa is replaced by columnar epithelium resembling intestinal tissue. This lesion of distal esophagus is associated with chronic GERD and predisposes patients to develop esophageal adenocarcinoma (EAC). However, the precise mechanism of development of BE and EAC is unclear. The incidence of EAC has been rapidly increasing in the last three decades and, interestingly, this increase correlates with the introduction of PPIs on market.

Epidemiological, animal, and clinical studies suggest that BE is formed in response to stress induced by two major components of refluxate: gastric acid and bile acids. Bile acids in combination with acid induce nitrosative stress, oxidative stress, DNA damage, and alterations in cell signaling. Furthermore, bile acids may induce the expression of proteins associated with phenotypic switch from normal squamous to intestinal phenotype such as Klf-4, villin, and CDX2.

PPIs, since their discovery in the late 1980s, have been largely used for treatment of acid related disorders, including BE. PPIs decrease secretion of gastric acid by inhibiting H^+/K^+ ATPase of parietal cells and alleviate symptoms associated with acid reflux. However, it is not clear if this therapy can completely suppress reflux of duodenal contents. Clinical studies using the Bilitec 2000® probe demonstrated that bile reflux is more common, and concentrations of bile acids are significantly higher in patients with BE than in patients with GERD. Furthermore, during the last five years, concerns have been raised, since long-term treatment with PPIs may produce adverse effects. In this review, we summarize the effects of PPIs on bile acids.

No treatment (pH ~2)

- Unconjugated and glycine-conjugated acids irreversibly precipate at normal stomach pH
- Taurine conjugated bile acids are soluble but they constitute only ~20% of total bile acids
- No bacterial overgrowth; no formation or secondary bile acids
- Bile acids are less concentrated
- Normal gastric emptying
- Normal gastrin levels

PPI (pH >4)

- Unconjugated and glycine-conjugated acids are soluble; they can interact with esophageal mucosa
- PPIs decrease overall stomach secretion and thus bile acids present in the stomach are more concentrated
- Bacterial overgrowth; bacteria induce formation of more toxic unconjugated bile acid
- Delayed gastric emptying
- Hypergasterinemia

Bile acids present in bile are conjugated with glycine or taurine, however there is small fraction of unconjugated bile acids in the normal bile. Glycine-conjugated bile acids represent more than 70% of all bile acid pool while taurine conjugated bile acids represent about 20%. Unconjugated bile acids are known to be more toxic compared to bile acids conjugated with glycine or taurine.

First, the stomach is normally free of bacteria, with the exception of *H. pylori*. PPI treatment is associated with bacterial overgrowth in the upper gastrointestinal tract, since the increase in pH creates environment more permissive for bacterial proliferation.[26,27] A majority of bacterial species residing in the stomach and duodenum may induce the deconjugation and dehydroxylation of primary bile acids to form more toxic, unconjugated, secondary bile acids, such as deoxycholic acid.[26,27] Indeed, studies show bacterial overgrowth and elevated levels of unconjugated bile acids in the gastric juice of patients treated with PPI.[28] The recent study of Yang *et al.* indicates that intestinal metaplasia of the distal esophagus is associated with alterations in the microbiome (a shift from a Gram-positive microbiome in normal esophagus to that of a Gram-negative anaerobic microbiome in inflamed/BE), however it is not clear from this study if patients were treated with PPIs.[28]

Second, the majority of bile acids (glycine conjugated and unconjugated) irreversibly precipitate at normal acidic environment of stomach (pH $\sim$2), thus they cannot cause cell damage or the alterations in cell signaling. In contrast, at higher pH (i.e., pH $>$ 4) glycine conjugated bile acids are soluble, unionized, and thus active since their pKa is about 4. Glycine conjugated bile acids are the most prevalent bile acids in human bile and thus increase of stomach pH by PPIs treatment to pH $>$ 4 may lead to the cellular damage by bile acids. Unconjugated bile acid have pKa $\sim$6.2, and thus they are are most active at this pH. In our studies, we have previously shown that acid, in combination with a bile acid cocktail that reflects the composition of bile acids present in the refluxate, induces oxidative DNA damage *in vitro* in different cell lines and *ex vivo* in esophageal biopsies.[29]

Third, since secretion of gastric acid in the stomach is reduced, then volume of secreted gastric juice is also low. Thus, concentration of bile acids refluxed into stomach and, consequently, to esophagus, is higher compared to the normal stomach where the secretion of gastric juices is high.

Fourth, PPIs may cause hypergastrinemia. Increased gastrin is linked to proliferation and cancer development.[30] Finally, PPIs have been shown to induce delayed gastric emptying, which may compromise the LES and increase the reflux of bile in the esophagus.

In summary, acid suppression therapy with PPIs may lead to the bacterial overgrowth and increased reflux of toxic, unconjugated bile acids. This may result in increased cell DNA damage, mutations, and, consequently, to BE and EAC development.

7. Should baclofen be added to PPIs to address nonacid and bile reflux factors?

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GERD, which is refractory to PPI therapy, is a frequently encountered clinical problem. This situation is most commonly, although not exclusively, found in patients with nonerosive reflux disease (NERD) in whom the response rate may be as low as 45%. In some cases of PPI refractoriness, the mechanism may be insufficient acid suppression by the PPI. This may be due to patient factors such as poor compliance or improper dosage time (i.e., not taken 30 min before mealtimes), or phamacokinetic/pharmacodynamic factors such as reduced PPI bioavailability, rapid PPI metabolism, or biological resistance to PPIs. These reasons may be best addressed by patient education, rationalization of medication regimes, and use of alternative acid-suppressant agents. However, a proportion of GERD patients have continued reflux-associated symptoms despite adequate efficacy of PPI therapy. These patients often have physiological esophageal acid exposure, yet when tested with 24-h multichannel intraluminal impedance (MII) studies, they have symptoms associated with reflux events that are not acidic, i.e., they may have sensitivity to reflux of other types. The refluxate in these cases may be weakly acidic (pH $>$ 4), and may contain bile components from DGER, or even gas.

Weakly acidic reflux as a cause of GERD symptoms

A study of 60 symptomatic GERD patients taking, and refractory to, PPI revealed that weakly acid

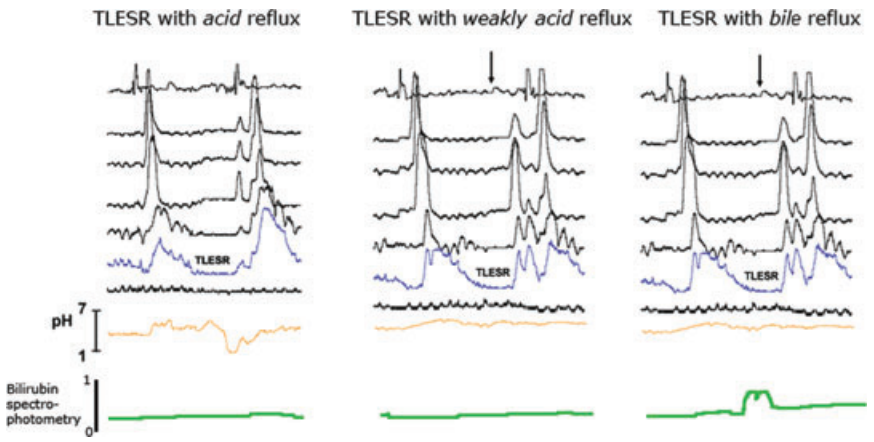

Figure 5. **Transient lower esophageal sphincter relaxations can be responsible for acid, weakly acidic, and bile reflux events.**

reflux was responsible for persisting regurgitation in 35%, cough in 20%, and heartburn in 7% of cases. Weakly acidic reflux appears to be of particular importance in NERD patients. Emerenziani *et al.* showed that 24% of GERD symptoms in NERD patients were attributed to weakly acidic reflux. There has been recent interest in the role of gaseous reflux symptom generation in GERD. Within their pH-MII study in GERD patients, Emerenziani *et al.* investigated the relationship between gaseous reflux (as measured by MII) and symptoms. They showed that in patients with NERD, the presence of gas with the liquid refluxate was associated with an increased probability of symptom perception. A similar study using pH-MII demonstrated that patients with NERD had an increase in gas-containing reflux episodes compared to controls. DGER is believed to be important in the genesis of a proportion of continuing symptoms despite PPI therapy, although the extent of its role remains controversial. Initial studies by the Leuven group suggested a significant role for DGER in PPI-refractory GERD. When patients with persistent reflux symptoms, despite therapy, were studied PPI, DGER (measured by esophageal bilirubin spectrophotometry) was found to be rather important, being related to 18% of symptomatic episodes versus 7% for acid and 10% for mixed reflux. Conversely, other studies have suggested a less important role for DGER in PPI-refractory GERD. Gasiorowska *et al.* studied a similar group of patients, and found DGER alone to be of a relatively lesser relevance, being related to 9% of symptom events versus 32% for acid and 32% for mixed reflux. Moreover, the amount of DGER in PPI-refractory patients was similar to that in PPI-responsive patients.

The TLESR as a therapeutic target

If weak acid, DGER, and gas can be responsible for GERD symptoms, it is perhaps not surprising that a proportion of patients remain refractory to PPI therapy. Although PPIs have been shown to reduce both acid and DGER, it would follow that for some patients an alternative therapeutic target to the proton pump should be addressed. The common factor associated with any reflux, be it acid, weak acid, DGER or gas, is the TLESR (Fig. 5). TLESRs are triggered in response to activation of stretch receptors in the stomach and are thought to be mediated by a vago-vagal reflex pathway. Gastroesophageal reflux almost always occurs in the context of a TLESR, and so the suppression of these relaxations is an attractive therapeutic target. This was first shown to be plausible by Mittal *et al.* in 1995 when atropine was shown to reduce both TLESR frequency and the number of reflux events in normal subjects.[31] Subsequently, $GABA_B$ agonists (an example of which is baclofen) have also been shown to reduce the rate of postprandial TLESRs and reflux episodes in humans. $GABA_B$ receptors act at several points along the vagal mechanoreceptor signaling pathway. Vela *et al.* studied nine healthy volunteers and nine symptomatic reflux patients with esophageal pH-impedance after both placebo and a single dose of baclofen. Baclofen was found to cause a significant reduction in both acid and nonacid reflux in all subjects.[32] The potential benefits of baclofen on DGER have also been shown. Koek *et al.* investigated

patients with PPI-refractory GERD who showed normal esophageal acid exposure, but who had pathological DGER on Bilitec monitoring. The addition of baclofen 20 mg three times daily to high-dose PPI therapy significantly reduced the number of DGER episodes, esophageal duodenal reflux exposure, and symptoms.[33] A further placebo-controlled study of 16 patients showed that 10 mg baclofen four times daily significantly reduced reflux events and improved symptoms.[34] Although effective at inhibiting TLESRs, the clinical use of baclofen is limited by its side-effect profile, causing troublesome symptoms such as dizziness and nausea. Recently, attention has turned towards new $GABA_B$-receptor agonists that act primarily at peripheral sites and, therefore, have better tolerability. The newly developed, peripherally acting $GABA_B$-receptor agonist lesogaberan has been shown to have a favorable safety and tolerability profile. Furthermore, in a randomized, double-blind, placebo-controlled trial in patients with PPI-refractory symptoms, lesogaberan significantly reduced TLESR frequency and number of reflux episodes compared to placebo.[35]

Summary

PPI-refractory GERD is a commonly encountered clinical problem. Before considering add-on therapy, reversible reasons for PPI failure (e.g., patient compliance) should first be sought. However, a subgroup of patients can be identified in whom ongoing symptoms are related to reflux events that are not acidic. In this group of patients the reduction of TLESR frequency is a potentially beneficial therapeutic strategy. $GABA_B$-receptor agonists have been shown to be effective in reducing acidic and weakly acidic reflux events, and can reduce DGER. Baclofen is a reasonable therapeutic add-on in those with persistent symptoms due to nonacid reflux in those who can tolerate it. The development of newer drugs with more favorable side-effect profiles is encouraging progress for the management of this difficult-to-treat group of patients. The search for the ideal TLESR-inhibiting drug remains ongoing.

8. In patients on PPI therapy, what is the threshold for adequate suppression of esophageal acid exposure as expected by pH monitoring?

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The normal esophageal pH values for healthy volunteers are well defined. However, many patients we study today are taking PPIs, and the degree of appropriate acid control on antacid medications has clinical relevance. Unfortunately, the threshold for adequate esophageal acid control on PPI therapy is poorly studied.

The single, widely quoted study addressing this question is from Brad Kuo, MD and Donald Castell, MD at Graduate Hospital, Philadelphia.[36] They studied 19 healthy male volunteers (mean age 25 years) assessing both esophageal and gastric pH in a randomized singled blinded protocol with three dosing arms: 40 mg omeprazole in the morning before breakfast, 40 mg omeprazole in the evening before dinner, and 20 mg twice a day before breakfast and dinner. Before each session, a baseline ambulatory pH study on each patient was done. Each

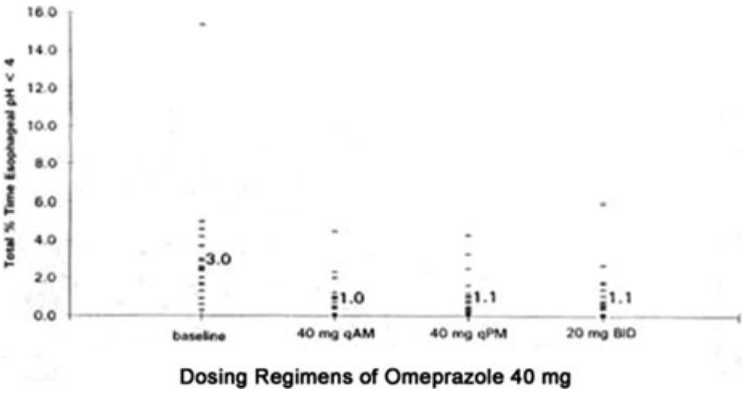

Figure 6. Dosing regimens caused a significant reduction ($P < 0.01$) in distal esophageal acid exposure compared with baseline.

session was carried out over at least five weeks with each dose session separated by at least a one-week washout period. Subjects received seven days of each dosing regimen followed by pH testing.

As shown in Figure 6, all dosing regimens caused a significant reduction ($P < 0.01$) in distal esophageal acid exposure compared with baseline. However, there was no significant difference among these dosing regimens with the range of means very tight at 1.0% to 1.1% for the total acid exposure time. The 95% confidence interval for the upper limit of acid exposure in these 19 volunteers across all studies was 1.6% on omeprazole 40 mg.

Using this threshold value of 1.6%, the same group performed a retrospective analysis of 45 patients with persistent GERD symptoms on omeprazole 20 mg BID.[37] Of this group, 14 (31%) had reflux that was poorly controlled based on the new threshold value of 1.6%. However, this separation did not characterize patients who would respond to increasing the omeprazole dose to 20 mg QID. Of the five patients with typical GER symptoms and pH >1.6%, three responded to the higher omeprazole dose. All three of the responders had a good symptom response on pH testing. In the ten patients (one overlapped with heartburn) with primarily atypical symptoms and pH >1.6%, only one patient with a good symptom correlation improved with QID omeprazole treatment. Thus, only 4 of 11 (36%) patients (three lost to treatment follow up in the atypical group) with pH thresholds >1.6% and persisted symptoms improved with higher doses of PPIs.

This data gives us little confidence in the proposed pH threshold <1.6% on PPI therapy and suggest that larger studies and possibly a multicenter study is required to appropriately address this issue. Until this time, our and most other groups, define adequate acid control in GERD patients as values below normal thresholds for healthy controls, usually % total acid exposure time of less than 4.5–5.5%.

9. What are the effects of PPIs on stabilization of basal cell proliferation, reduction of cell cycle abnormalities, and hyperplasia of the basal cell layer?

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The effects of PPIs on esophageal mucosa are dependent on the type of epithelium present. Physiologically, the esophagus is covered by squamous epithelium. When the squamous epithelium is replaced by columnar epithelium (BE), effects on columnar epithelium can be observed as well.

Squamous epithelium

The esophagus is physiologically lined by nonkeratinized squamous epithelium. Proliferation (regeneration) starts from the basal cell layer. Stromal papillae that extend into the epithelium mainly contain small capillaries. In reflux disease, increased proliferation leads to thickening of the basal cell layer and elongation of stromal papillae (Fig. 7). Capillaries are now located closer to the mucosal surface, and this corresponds to increased redness of the mucosa during endoscopy. At high magnification endoscopy dilated capillary loops may be seen.

The thickness of the basal cell layer and the length of the papillae can be expressed as percentages in relation to the thickness of the entire squamous epithelium. A relationship between these percentages and the severity of disease may be noted: the more severe the reflux disease (according to the Los Angeles classification), the more pronounced the thickness of the basal cell layer and the length of the papillae. After PPI therapy these values drop down markedly. Even if missing or poor controls do not allow a precise definition of what is normal and what is already abnormal, it may be speculated that normal values are very close by to these values induced by PPI therapy (Fig. 8). Since under PPI the length of papillae decreases, capillaries move further away

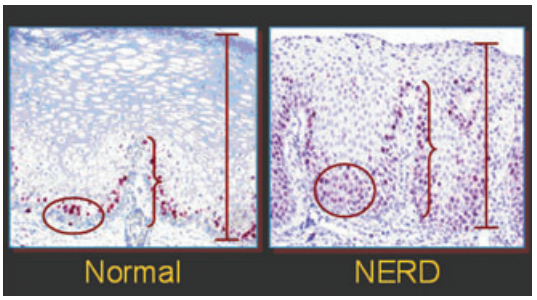

Figure 7. **Histology of esophageal squamous epithelium in an individual without pathological changes (left) and an individual with endoscopy negative reflux disease (NERD) (right). Proliferating cells are marked immunohistochemically by Ki67. Circle = basal cell layer; brackets = length of stromal papilla; distance holder = epithelial thickness.**

from the lumen leading to a decreased redness of the mucosa up until virtual normalization during endoscopy.[38]

Columnar epithelium

Columnar metaplasia of the distal esophagus (BE) is regarded as a precancerous condition. The risk for malignant transformation is up to 125 times higher than in the normal population. Cancer risk can also be expressed as 0.5% per year for individuals with BE. How to decrease this risk? One option is to remove the esophagus or at least part of it by surgery. This approach would be linked to numerous procedure-related side effects. The question is whether PPI therapy is capable of lowering the risk of malignant transformation, since PPI therapy is known to decrease proliferation, enhance differentiation, and promote epithelial maturation. Not many publications are available on this topic and the study design of the few reports available is limited. It appears, however, that PPI therapy reduces the risk of malignant transformation.[39–42] NSAIDs are thought to cause a trend only, but no significant reduction on its own. Of note, several reports stressed that the effects of PPI therapy may be related to different profiles in acid exposure. Namely there are differences in continuous versus pulse acid exposure. The epithelium of patients with continuous acid exposure is believed to only partially benefit from PPI therapy, whereas the epithelium of patients with pulse acid exposure, leading to more pronounced epithelial changes, might have a greater potential to be positively influenced/cured by PPI therapy.

It needs to be stressed that these data, although corresponding nicely with our expectations, are so far only limited, based upon few (observational) studies with few tested individuals, sometimes biased by poor study design (such as decreased bile reflux under PPI-therapy), or even nonreproducible. Thus, although the mechanisms have not yet been fully understood, at least a trend to risk reduction during PPI-therapy is accepted in the literature.

Length regression of BE has been known for a long time,[43] but to be significant it should exceed 10% of the area covered by columnar epithelium. Especially in short segments this cannot properly be accessed by endoscopy since, for example, 10% of 3 cm would sum up as only 3 mm in length reduction. Again only a trend has been documented so far even if Barrett's will not disappear completely in most cases.

In conclusion, established PPI effects on squamous epithelium are:

1. increase of squamous islands;
2. reduction of cell proliferation;
3. improvement of differentiation;
4. reduction of acid reflux;

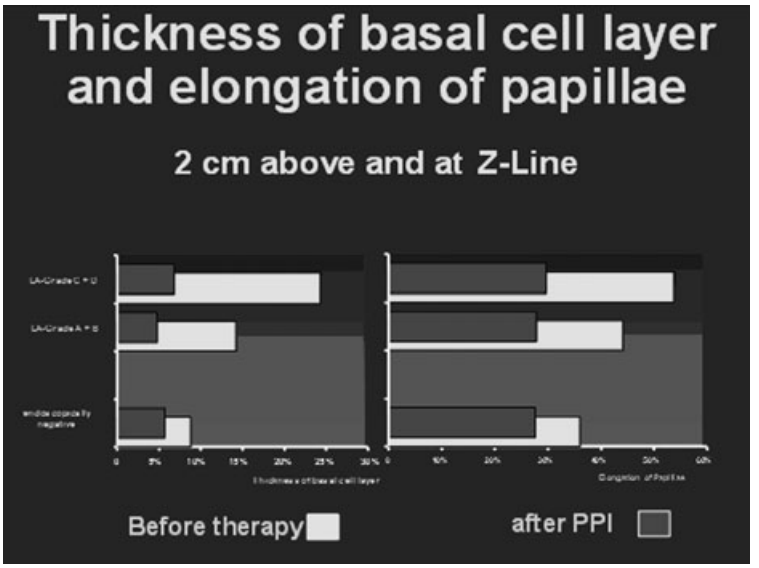

Figure 8. **Changes in esophageal squamous epithelium after PPI therapy focusing on length of stromal papillae and thickness of basal cell layer related to severeness of reflux disease assessed by Los Angeles classification (LA Grade). Modified after Vieth *et al.* [38]**

5. ERD healing improves detection of neoplasia;
6. adjuvant treatment to ablation therapy; and
7. symptom control.

PPI effects on columnar epithelium are partially based on much weaker evidence:

1. regression of Barrett's length (10% !);
2. reduction of cell proliferation (?);
3. improvement of differentiation (?);
4. decreased COX-2/VEGF expression and PGE2 release;
5. reduction of bile reflux (?);
6. ERD healing improves detection of neoplasia;
7. adjuvant treatment to ablation therapy; and
8. symptom control.

10. Can PPI therapy achieve normalization of esophageal pH in Barrett's patients?

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The use of PPIs is almost universal in patients with BE. These drugs effectively control reflux symptoms; heal mucosal damage; prevent recurrent esophagitis and/or stricture formation; partly regress the metaplastic surface; induce formation of neosquamous islands; reduce DEGR, minimizing the impact of bile on the metaplastic epithelium; facilitate the recognition and regression of dysplasia; and may prevent dysplasia and adenocarcinoma by decreasing cell proliferation, exerting potentially a chomoprevention effect. In this context, maximum esophageal pH control (esophageal pH normalization) is generally considered as essential. PPI therapy is also an important adjuvant treatment to ablation modalities, facilitating neoepitheliazation.[44]

Figure 9A shows an example of "ideal" pH control in BE, where the esophageal pH was normalized to pH < 4.0 for less than 4% using esomeprazole 40 mg bid, and partial suppression of intragastric acidity was accomplished. In contrast, Figure 9B shows "poor" pH control in BE where the esophageal pH remained persistently abnormal despite esomeprazole 40 bid; partial and inadequate suppression of intragastric acidity, particularly at night was noted. In one study, 62% of patients failed to achieve complete esophageal acid suppression (24-h pH < 4.0 for $> 5\%$ time) despite esomeprazole 40 mg bid; typically esophageal pH reflects gastric pH, particularly at night (supine position).[45] In another "dose response" study with esomeprazole, 31 patients with BE were noted to accomplish gastric pH >4.0 for 88.4%, 81.4%, and 80.4% of day 5

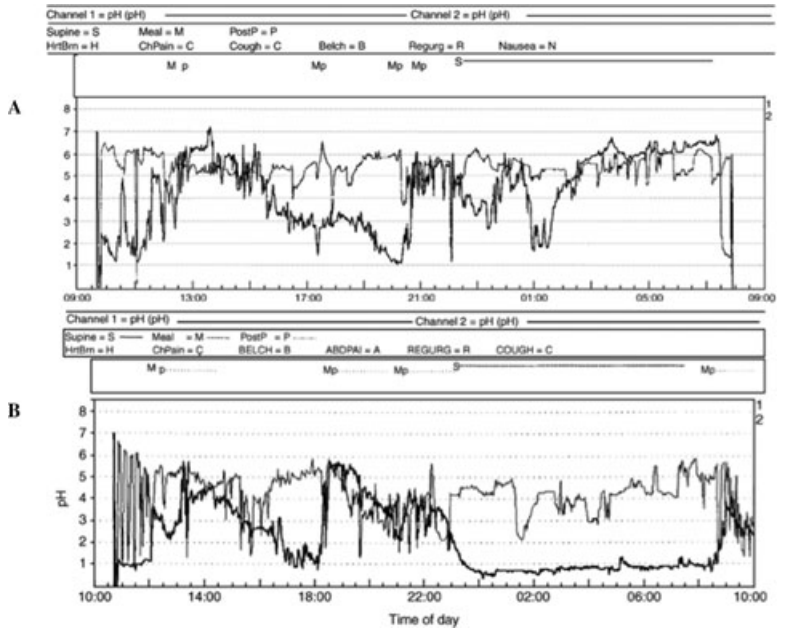

Figure 9. (A) "Ideal" pH control in Barrett's esophagus: esophageal pH is normalized to pH <4.0 for less than 4%; partial suppression of intragastric acidity. (B) "Poor" pH control in Barrett's esophagus. Esophageal pH is persistently abnormal despite esomeprazole 40 bid; partial and inadequate suppression of intragastric acidity (originally published in Ref. 45).

after treatment with esomeprazole 40 mg tid, 40 mg bid, and 20 mg tid, respectively. The esophageal pH remained abnormal in 16%, 23%, and 19% of patients receiving esomeprazole 40 mg tid, 40 mg bid, and 20 mg tid, respectively.[46]

In a crossover trial, there were no significant differences among three PPIs (lansoprazole, omeprazole, and esomeprazole) in controlling esophageal pH. Although all these patients had no GER symptoms, many exhibited persistently abnormal esophageal pH.[47] There are no predictors of normalization of esophageal acid exposure in BE patients. In a cohort study of 46 patients with BE, 25% continued to have abnormal esophageal pH profiles despite bid PPI, and age, BE length, and hiatal hernia size did not predict the persistence of abnormal intraesophageal pH.[48] It appears that acid remains in the esophagus of Barrett's patients because of poor peristalsis of the esophageal body, and most of the reduction in esophageal pH exposure with PPI happens in the upright, not supine position. Similarly, upright—not supine—duodeno-gastro-esophageal (bile) reflux was significantly reduced by PPI in such patients.[49]

In conclusion, a high percentage of patients with BE continue to exhibit pathologic GERD and low gastric pH despite high (bid-tid) doses of PPI and all PPIs have a similar effect. For an antisecretory treatment aimed at chemoprevention to be effective, higher PPI dosing, confirmed by pH monitoring, is necessary.

11. Are different effects of PPI therapy to be expected in relation to the predominant phenotype of cellular proliferation in Barrett's epithelium?

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The chemopreventive effect of PPIs for BE has been extensively studied; however, its role remains unclear. Epidemiologic studies suggest a protective effect of PPIs against neoplastic changes and progression in BE; however, the data are contradictory. It is well known that BE consists of two secreted mucin phenotypes, namely gastric and intestinal. The predominant mucin phenotype has been reported to have a more malignant potential. Consistently, we have shown that Barrett's epithelium with intestinal predominant mucin phenotype showed markedly elevated cellular proliferation and suppressed apoptosis, as compared to that with gastric predominant mucin phenotype.[50,51] Therefore, the controversy regarding the chemopreventive potential of PPI, including the suppression of COX-2 expression and cell proliferation, and the induction of apoptosis, may be the diversity of the mucin phenotype of Barrett's mucosa. The aim of this study[52] was to evaluate the effect of PPIs on cellular proliferation, COX-2 expression, and apoptosis in BE with different mucin phenotypes.

Materials and methods

Four hundred and nineteen consecutive patients with histologically proven BE were enrolled in the study.[52] Patients were divided into two groups, nontreatment patients ($n = 358$), and chronic PPI users ($n = 61$), which were defined when they were continuously administrated from at least two months before endoscopy. Four hundred and sixty-six biopsy samples of BE from 358 nontreatment patients and 81 from 61 chronic PPI users were immunohistochemically examined using anti-COX-2 protein, antiproliferating cell nuclear antigen (PCNA), and antisingle strand DNA (ssDNA) antigens in both mucin phenotypes of BE.

Results

Among nontreatment patients and PPI users, there was no significant difference between the gastric and intestinal predominant mucin phenotype.[52] Prevalence of the COX-2 expression pattern did not significantly differ between the nontreatment and PPI users. In those using PPIs, significant suppression of cellular proliferation assessed by PCNA was found in BE with the gastric predominant mucin phenotype, but not with the intestinal predominant mucin phenotype. Apoptosis indices assessed by ssDNA in chronic PPI users did not significantly differ between the two mucin phenotypes.[52]

Discussion

Acid exposure is considered the main factor not only in the development of BE, but also in the carcinogenesis of Barrett's adenocarcinoma. In this study, we have demonstrated that PPIs showed an inhibitory effect on cellular proliferation only in the gastric predominant mucin phenotype but not in the intestinal predominant mucin phenotype showing more accelerated cellular proliferation activity (Fig. 10).[52]

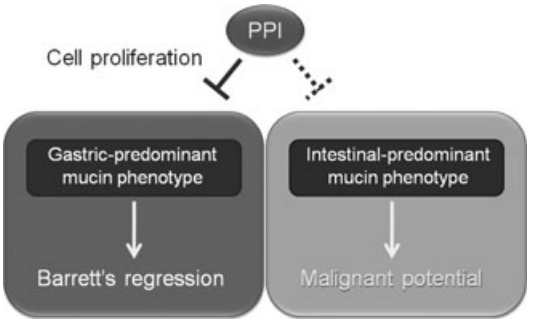

Figure 10. **The effect of PPI on cell proliferation in BE.**

In BE with the intestinal-predominant mucin phenotype, COX-2 expression and subsequent possible overproduction of prostaglandins is closely related with the acceleration of cellular proliferation and the inhibition of apoptosis. However, the suppressive effect of PPIs for COX-2 expression in BE was not found in this study.

Conclusion

PPIs suppress cellular proliferation in BE with the gastric-predominant mucin phenotype but not in that with the gastric-predominant mucin phenotype. This finding may at least partly explain the ongoing controversy surrounding the notion that all cases of BE respond to acid-suppressive therapy.

12. What are the respective values of the Symptom Index, the Symptom Sensitivity Index, and the Symptom Association Probability for the interpretation of causality of symptoms, and the potential response to medical treatment?

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The first symptom indices to relate acid reflux by ambulatory pH testing with patients symptoms evolved in the late 1980s.[53] The "symptom index" (SI) is defined as the percentage of symptom episodes that are related to reflux:

Number of reflux related symptom episodes/
Total number of symptom episodes × 100%.

The distribution of SIs in a population of patients with heartburn appears to be bimodal, and the result of receiver operating characteristic analysis indicates that the optimal threshold for SI is 50%. The major shortcoming of the SI is that this index does not factor in the total number of reflux episodes, hence the higher the frequency of acid reflux, the greater the likelihood that a symptom is associated by chance. For this reason the "symptom sensitivity index" (SSI) was proposed as an additional parameter. SSI is defined as:

Number of symptom associated reflux episodes/
Total number of reflux episodes × 100%.

SSI values of 10% or higher are considered to be positive.[53] It should be noted that these determined thresholds have only been validated for heartburn and regurgitation—not for chest pain or extraesophageal symptoms. Furthermore, these indices have not been validated for nonacid episodes. Both the SI and SSI suffer from the disadvantage that they do not integrate all factors determining the relationship between symptoms and reflux. The "symptom association probability" (SAP) is calculated by dividing 24-h pH data into consecutive two minute windows.[54] For each of these two minute windows, it is determined whether reflux occurred and whether symptoms occurred, giving a 2 × 2 contingency table of S+R+, S–R+, S+R–, S–R–, respectively. Fishers' exact test is used to calculate the probability (p) that the observed distribution could occur by chance alone. SAP is calculated as $(1 - p) \times 100\%$; by statistical convention, SAP $\geq$ 95% is positive. Limitations of validation outside classical GERD symptoms also apply to the SAP.

Despite the availability of these symptom indices, there is surprisingly little literature assessing their predictive accuracy in a clinical population. To perform this appropriately, a large patient population needs testing with outcomes followed after aggressive PPI therapy or antireflux surgery. The only study to date taking this approach was performed by Taghavi *et al*. from Iran.[55] The authors studied 52 patients with a predominant symptom of heartburn with baseline symptom scores calculated at the first visit. After 24-h pH testing off all PPIs, the symptom reflux indices were calculated. All patients were placed on high dose omeprazole (40 mg AM, 20 mg at night) and symptom scores recorded again one week later. A reduction of >50% in the heartburn score was considered a positive omeprazole test. Overall, the omeprazole test was positive in 23 patients (52%). The concordance of the three symptom indices and the omeprazole test

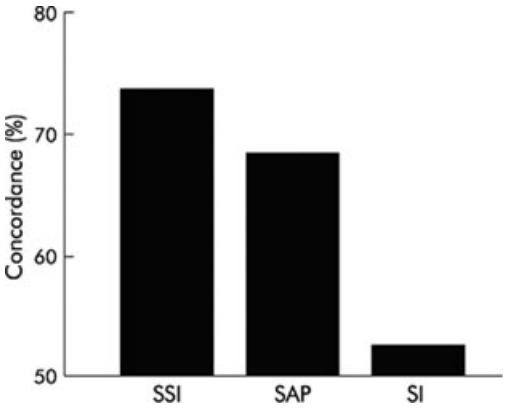

Figure 11. **Concordance of the three symptom indices and the omeprazole test.**

is shown in Figure 11. All these indices were significantly related to each other ($P < 0.001$). However, only the SAP and SSI had a statistically significant relationship with the omeprazole test ($P < 0.05$ for both). SSI had the highest positive (80.9%) and negative (57.9%) predictive values and sensitivity (73.9%). The specificity of the SSI and SAP (73.3% for both) was lower than the SI (80%). These authors could find no cutoff point at which the results of SI could be related significantly to the omeprazole test results. The authors concluded, "these symptom reflux indices are unable to predict the response of the one most typical symptom of GERD (heartburn) to the one most effective medical treatments available."[55]

No other studies have been reported to date to address this issue in individual patients. However, other studies have shown favorable trend data for both the SAP and SI when assessing the response for heartburn and regurgitation to PPI therapy over several weeks.[56,57] In these studies, those with a positive symptom index and abnormal reflux values performed as a group best, followed by a positive symptom correlation even with a normal pH test, while not surprisingly those with both tests negative did the worst. Based on these limited data, great caution is urged when using these test results to predict a response to PPIs in individual patients, especially those without classic heartburn or regurgitation. Large, possibly multicenter studies need to be performed using strong clinical endpoints such as symptom resolution to prolonged high dose PPI therapy (BID dose for four to eight weeks) or antireflux therapy. Additional studies are also required to validate the use of these indices with nonacid reflux measured by impedance technology.

13. Can correlation between use of PPI and development of dysplasia be considered?

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Use of PPIs may be useful to avoid the development of dysplasia if there are not concomitant duodenogastroesophageal reflux (DGER) and other systemic factors interacting, and if it could really normalize esophageal acid exposure.

Reflux symptoms can be controlled in most patients with PPI therapy. Twice a day dosing may be necessary in a subgroup of patients. However there are currently no data that directly support the use of high dose antisecretory therapy to delay or prevent the development of Barrett's esophagus or EAC. Even on higher doses of omeprazole of 40 mg bid, as many as 24% of Barrett's patients were shown to have abnormal total or supine distal esophageal acid exposure values. Limited prospective studies have demonstrated normalization of esophageal pH with q.d. PPI therapy in over 90% of patients with typical reflux symptoms. On the other hand, patients with more severe degrees of erosive esophagitis have significantly greater abnormal esophageal acid exposure in spite of PPI therapy.[58]

Furthermore, adequate acid suppression may mask the detection of nonacid reflux events. PPIs, although effective in controlling the acid component of the refluxate, do not eliminate the reflux of bile, which some believe to be a major contributor to the pathogenesis of Barrett's epithelium. DGER occurs in more than 50% of patients with erosive esophagitis and BE,[59] and the majority of bile reflux events occurred concomitantly with acid reflux. Tack *et al.*[60] have recently published a series of studies suggesting a possible role for DGER in both symptoms and esophagitis in a subset of patients with difficult to manage, symptomatic reflux. Surprisingly, 51% of patients had erosive esophagitis on endoscopy despite the fact that they were on PPI therapy at the time of the study.[60]

Chronic inflammation seems to have a central role in the development of esophageal adenocarcinoma and its precursor lesions. The refluxate contains numerous substances in addition to gastric

acid, including bile salts, pancreatic enzymes, and ingested foods and their metabolites, which can cause acute and chronic inflammation of the oesophageal epithelium with resulting oxidative stress.

Abdominal obesity, in addition to promoting gastroesophageal reflux, is also increasingly being recognized as causing a state of low-level systemic inflammation, characterized by increased plasma levels of proinflammatory cytokines and receptors, such as interleukin-6 (IL-6), TNF-α and TNF-α receptor 2, C-reactive protein and leptin.

In addition, cigarette smoking can cause inflammation both systemically and in the esophageal epithelium in response to swallowed smoking products. In turn, a chronic state of systemic and localized inflammation and oxidative stress promotes DNA damage, cellular proliferation and telomere shortening, which can increase the risk of developing clones containing small- and large-scale genomic alterations, eventually leading to widespread chromosomal instability and esophageal adenocarcinoma.

The advisability of prescribing aggressive antireflux therapy for all patients with BE, irrespective of the severity of their underlying GERD, has been debated. One recent study has shown a decrease in development of dysplasia in patients treated with or prescribed PPIs.[61] In multivariate analysis, the use of PPI after BE diagnosis was independently associated with reduced risk of dysplasia, hazards ratio: 0.25 (95% CI 0.13–0.47), $P < 0.0001$. In this same route, a retrospective observational study of 344 patients with BE, PPI treatment after diagnosis of BE was associated with a reduced risk of HGD or cancer.[62]

On the other hand, several uncontrolled, observational studies have found fewer cases of dysplasia and cancer among patients with BE who had antireflux surgery than among those who had received medical treatment, and some even have proposed that antisecretory therapy might predispose to malignancy. However, the limited studies that have addressed this issue directly have not found a significant association between esophageal adenocarcinoma and the use of antisecretory agents *per se*.

Conflicts of interest

The authors declare no conflicts of interest.

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