J. Clin. Biochem. Nutr., **42**, 71–75, March 2008

Pleotropic Effects of Proton Pump Inhibitors
Guest Editor: Yuji Naito

Proton Pump Inhibitors and Gastritis

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Received 31 October, 2007; Accepted 9 November, 2007

Summary **Proton pump inhibitors (PPIs) are novel compounds that strongly inhibit the H^+/K^+-ATPase in the gastric parietal cells to cause profound suppression of acid secretion. Acid-generating ATPase, also known as vacuolar-type ATPase, is located in the lysozomes of leukocytes and osteoclasts and its activity is also reportedly influenced by treatment with PPIs. This concept is supported by the results of studies using autoradiography in which ^{3}H-Lansoprazole uptake sites were clearly detected in the cytoplasmic granules of neutrophils infiltrating the gastric mucosa. *In vitro* studies indicate that PPIs increase the intra-vacuolar pH in the lysosomes of purified neutrophils and attenuate the adherence of neutrophils to the vascular endothelium. In clinical practice, the acidic environment in the stomach plays a critical role in the development of gastritis induced by *Helicobacter pylori* (*H. pylori*). This is worthy of note, because persistent gastritis often results in atrophic and metaplastic changes in the gastric mucosa, which are believed to be preneoplastic abnormalities. In patients with *H. pylori*-infection, PPI therapy causes corpus-predominant gastritis, which is frequently found in the background mucosa in patients with gastric cancer. The efficacy and safety of long-term PPI-treatment have not been conclusive, thus we need to pay more attention to the additional pharmacological actions of PPIs.**

Key Words**: gastritis, *Helicobacter pylori*, vacuolar H^+-ATPase, osteoclast, leukocyte**

Introduction

Gastric H^+/K^+-ATPase is a key proton pump that exchanges hydrogen (H^+) and potassium (K^+) ions across the canalicular membrane in acid-secreting parietal cells. Proton pump inhibitors (PPIs) (omeprazole, lansoprazole, rabeprazole and pantoprazole) are initially absorbed *via* the small intestine and distributed to the gastric parietal cells, where they are activated in the acidic environment of their acidic vesicles and secretory canaliculi. Profound acidity ($pH < 1$) is reportedly necessary for the activation of PPIs, thus it has been believed that parietal cells are the only sites in the human body in which PPIs are activated. However, cells such as leukocytes and osteoclasts are also known to have acidic vesicles in their cytoplasm. The pump in these cells is known as vacuolar-type H^+-ATPase, different from gastric H^+/K^+-ATPase (Table 1). If PPIs indeed exerted

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Table 1. Characteristics of acid-generating ATPase

	Parietal cell H^+/K^+-ATPase	Vacuolar H^+-ATPase
Location	Stomach	Osteoclasts
	(parietal cell)	Leukocytes
		Kidney epithelial cells
Physiological role	Secretion of gastric acid	Bone resorption
		Phagocytosis
		Acidification of urine
Potent inhibitor	Proton pump inhibitor	Bafilomycin A1
Level of acidity (pH)	<1	3–6

Table 2. Effect of omeprazole (OPZ) on the intralysosomal pH in purified human neutrophil as estimated from the FITC-fluorescence ratio. Data indicate mean ± SD. *p<0.05 and **p<0.001, compared with the control value. Reprinted with permission [*5*]

	Control (n = 12)	OPZ 10^{-6} M (n = 12)	OPZ 10^{-5} M (n = 12)	OPZ 10^{-4} M (n = 12)
Fluorescence ratio (495 nm/420 nm)	0.245 ± 0.017	0.280 ± 0.018	0.303 ± 0.023*	0.351 ± 0.019**
Estimated intralysosomal pH	5.81 ± 0.09	6.00 ± 0.09	6.12 ± 0.12*	6.37 ± 0.10**

effects on the functions of these cells, additional interesting therapeutic possibilities may be revealed for PPIs. However, we would also need to pay attention to the adverse effects of long-term and high-dose PPI therapy.

More than half of the Japanese population is reported to be infected with *Helicobacter pylori* (*H. pylori*). Unfortunately, Japanese National Health Insurance does not cover treatment of asymptomatic *H. pylori* infection, even though gastric cancer has clearly been demonstrated to be associated with *H. pylori* infection. Corpus-predominant gastritis is known as a high-risk gastritis pattern in relation to the development of gastric cancer, and is sometimes promoted by treatment with antisecretory drugs.

Effects of PPIs on Cellular Functions

The actions of PPIs on the functions of cells other than parietal cells have not been well studied. Wandall *et al.* [*1*] reported that omeprazole exerts effects on neutrophil chemotaxis, degranulation and superoxide production. It has also been demonstrated that oxygen-derived free radical production from activated neutrophils is inhibited by lansoprazole *in vitro*, and by an autoradiographic study, that ^{3}H-lansoprazole-binding sites are present in the cytoplasmic granules of neutrophils infiltrating the gastric mucosa; also, omeprazole was reported to attenuate the adherence of neutrophils to the venular endothelium of the rat mesentery [*2*, *3*]. Yoshida *et al.* [*4*] reported that expression of integrins (CD11b and CD18) in activated neutrophils was attenuated by omeprazole and lansoprazole. The molecular mechanisms by which PPIs affect the neutrophil functions have not yet been fully elucidated. NADPH-oxidase (oxidant-producing enzyme) and integrins (adherence molecule) are membrane-bound enzymes that are activated by fusion of the lysosomal membrane. Acidification of lysosomes is also achieved by fusion of the cytoplasmic vesicles. Thus, the effects of PPIs on the functions of neutrophils may be associated with lysosomal dysfunction. To explore the underlying mechanisms, we examined the effects of PPIs on the lysosomal pH of purified neutrophils activated by f-methionyl-leucyl-phenylalanine (FMLP) and opsonized zymosan by assessing the fluerescence intensity ratio of phagocytosed FITC-dextran using digital-fluorescence video microscopy. The results revealed that the lysosomal pH increased in a dose-dependent manner by pretreatment with omeprazole (1–100 μM) (Table 2) [*5*]; the intralysosomal pH in the absence of omeprazole was 5.81, which was not sufficient for activation of omeprazole, because the pKa of omeprazole is about 4.0, while the pH in the cytoplasmic vesicles that subsequently translocated to secondary lysosomes was estimated to be more acidic (pH < 3). If leukocyte dysfunction is evoked by PPIs, the bactericidal functions of the cells may be affected. This possibility is supported by a recent clinical report indicating that the prevalence of pneumonia was significantly increased in a subject population administered PPI therapy [*6*].

An acid-secreting proton pump is also expressed on the plasma membrane of bone-resorbing osteoclasts, and also of macrophages and kidney epithelial intercalated cells [*7–9*]. This pump mediates the acidification of the extracellular environment and belongs to the family of vacuolar-type H^+-ATPases [*10*]. Visentin *et al.* [*11*] reported that a novel

selective inhibitor of the osteoclastic v-type ATPase prevented bone loss in rats. Although the osteoclast v-type ATPase is different from the parietal H^+/K^+-ATPase, Tuukkanen *et al.* [*12*] reported that omeprazole also inhibits bone resorption *in vitro*. In a trial conducted by Mizunashi *et al.* [*13*], omeprazole changed bone resorption parameters, such as the urinary hydroxyproline and calcium levels, suggesting that it can suppress bone resorption. These data indicate the possible efficacy of PPIs in the treatment of osteoporosis to prevent bone fractures. However, a recent case-control study demonstrated that the risk of hip fracture was increased in patients on long-term PPI therapy [*14*]. These results suggest that the potentially protective effect of osteoclatic proton pump inhibition may attenuate some of the negative effects of gastric acid suppression of PPIs, including on calcium absorption.

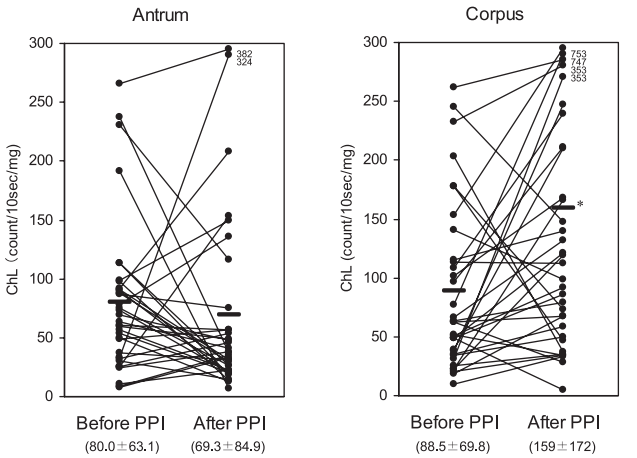

Fig. 1. Effect of proton pump inhibitors (PPIs) on the luminol-dependent chemiluminescense (ChL) activity in *H. pylori*-infected patients (n = 34). The antral ChL remained unchanged after PPI treatment, while a significant increase was observed in the corpus ChL value. **p*<0.02, compared with the pretreatment value. Reprint with permission [*22*].

Clinical Implications of PPI Treatment on the Development of Gastritis

The effects of PPI treatment on mucosal inflammation in the stomach are quite complex. The severity of gastritis is simply influenced by the acidity of the environment and leukocyte activity, and both are attenuated by PPIs, as described before. It is generally accepted that *H. pylori* is the most commonly encountered pathogen in cases of gastritis. PPIs reportedly inhibit not only the bioactivity of *H. pylori*, but also its urease activity which is important for bacterial survival in the acidic environment [*15*, *16*]. These *in vitro* observations are supported by clinical evidence: it was demonstrated that the whole intragastric urease activity as assessed by the urea breath test (UBT), was attenuated by treatment with PPIs [*17*]. However, Logan *et al.* found that treatment of *H. pylori*-infected gastritis with PPIs (omeprazole, 4 weeks) improved the histological severity of gastritis in the antrum, but not in the corpus [*18*]. This observation is compatible with the author's data that the myeloperoxidase content, a quantitative measure of neutrophil infiltration, decreased in the antral mucosa, but significantly increased in the corpus mucosa in patients with *H. pylori*-infected antrum-predominant gastritis [*19*]. Interestingly, the bacterial density of *H. pylori* decreased in the antrum, but remained unchanged in the corpus after PPI therapy. This observation implies that the aggravated corpus gastritis was not due to increased bacterial intensity. Although the mechanism is still unclear, several possibilities are postulated. The first possibility is involvement of the chemotactic molecule, monochloramine. Monochloramine is synthesized by the reaction of ammonia with hypochlorite derived from activated neutrophils [*20*], and its synthesis is promoted as the pH rises. Thus, treatment with PPIs may aggravate monochloramine-dependent corpus gastritis. The second possibility is closer contact between the bacteria and the gastric mucosa sensitive to immune inflammatory responses; deep invasion of *H. pylori* into the gastric pits may evoke strong inflammatory reaction. However, little evidence supporting this phenomenon has been reported so far.

Kuipers *et al.* [*21*] reported the important finding that atrophic gastritis was promoted in *H. pylori*-infected patients treated with omeprazole for a long time. Since atrophic change of the gastric mucosa has been believed to be a precancerous lesion, this report has attracted much attention. It is acknowledged that atrophy is the final stage of mucosal inflammation, thus progression to atrophy is associated with the enhanced inflammation evoked by PPI treatment. Authors found that 8-week treatment with PPIs elicited a 2-fold increase in oxidative stress in the corpus mucosa, although only a 10–20% increase was observed in the mucosal contents of myeloperoxidase and IL-8 (Fig. 1) [*22*]. Oxidative stress was further increased after long-term (24 weeks) treatment with lansoprazole [*23*]. Corpus-predominant gastritis is known as a high-risk factor for the development of gastric cancer [*24*]. Thus, we must pay attention to the risk of carcinogenic mutations in *H. pylori*-infected patients receiving PPI therapy. No systematic clinical trials have been performed to elucidate the relative cancer risk of PPI therapy in *H. pylori*-infected subjects. Hypergastrinemia, which is frequently induced by PPIs, is thought to be another potent risk factor for carcinogenesis. Thus, we need to carefully follow the changes in the gastric mucosa after PPI administration unless anti-*H. pylori* therapy is successful. The efficacy of PPIs on non-*H. pylori*-related mucosal inflammation remains unclear. It has been

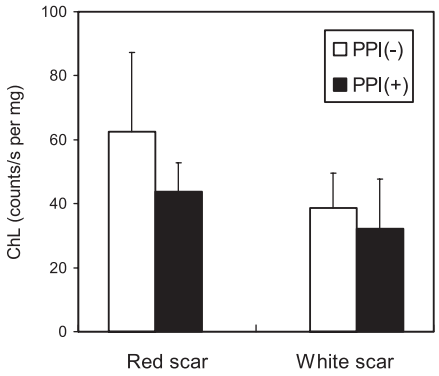

Fig. 2. Luminol-dependent chemiluminescense (ChL) activity in the red scars of ulcers and white scars tissue of ulcers in the gastric mucosa after successful *H. pylori* eradication. Patients who were prescribed PPIs exhibited lower ChL values than patients who did not receive PPIs after the eradication therapy.

demonstrated that treatment with lansoprazole reduces the mucosal inflammation in esophagus with gastro-esophageal reflux disease (GERD) [*25*, *26*]. The authors found that the oxidative stress in the ulcer scar portion in *H. pylori*-negative patients was significantly decreased in patients treated with PPIs as compared with that in patients not treated with PPIs (Fig. 2) [*27*]. Ulcer relapse is associated with focal inflammation, therefore PPI therapy may be useful to promote ulcer healing in *H. pylori*-negative subjects.

Conclusions

PPIs may modulate not only gastric H^+/K^+-ATPase activity, but also v-type H^+-ATPase activity, which is widely distributed in a variety of cells in the human body. Among these, the acid-producing systems in osteoclasts and leukocytes are well developed for maintaining bone turnover and exhibit bactericidal roles and promote tissue-destructive inflammation. Therefore, there is still much potential for research on the pharmacological and clinical aspects of PPI treatment.

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