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Pleiotropic Effects of Proton Pump Inhibitors
Guest Editor: Yuji Naito

Lansoprazole Novel Effector Sites Revealed by Autoradiography: Relation to *Helicobacter pylori*, Colon, Esophagus and Others

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Summary **Lansoprazole uptake sites by two kinds of autoradiographic procedures were compared with recent literature. The uptake sites have been seen in the *Helicobacter pylori*, colonic epithelial cells, inflammatory cells, peripheral autonomic nerves and enterochromaffinlike cells as well as gastric parietal cells. Each uptake sites corresponded to the reported localization of P-type ATPase or acidic compartment.**

***Key Words*: lansoprazole, autoradiography, *Helicobacter pylori*, colon, inflammatory cell**

Introduction

In almost all biological system, ATP is used as an energy source. As most cells are impermeable to ATP, it must be recycled in each cell. As a consequence, every cell catalyses an ATP synthesis/ATP hydrolysis cycle. A diverse ATPases runs this system; at least three classes are ion motive ATPases; phosphorylated (P), vacuolated (V) and F_0F_1 (F) types [*1*].

The gastric H, K-ATPase is a member of the P-type, ion-motive ATPase gene family. In eukaryotes, other members of the family are the Na, K-ATPases, the Ca ATPases of sarcoplasmic reticulum, endosome and plasma membrane, and the fungal H-ATPases [*2*, *3*].

As to the H, K-ATPase, it is clear that the expression of the genes encoding the two subunits of the gastric H, K-ATPase is not restricted to the stomach. Furthermore, a number of closely related H, K-ATPase isoforms have been discovered. These isoforms are also expressed in several organs including distal colon and distal tubule of kidney [*4*].

Substituted benzimidazoles, omeprazole, lansoprazole, pantoprazole and rabeprazole have been one of the World's top selling drugs because of its effectiveness to gastroduodenal ulcers, gastroesophageal reflux diseases, specificity of the effector sites and safety. Its specificity is based on the characteristics that this agent is a prodrug of sulfenamide and accumulates in the acidic compartment and converted to the rather impermeable sulfenamide and binds covalently to cysteine accessible from the extracytoplasmic face of the enzyme [*5*]. This means that acidic environment with cysteine residues in the luminal surface could be novel targets of this agents and their derivatives.

We have performed the autoradiography using tritiated lansoprazole for the last twelve years. This paper was attempted to review recent publications related to ATPases and substituted benzimidazoles and clarify the significance of our autoradiographic observation.

Autoradiographic Procedures Used in This Study

To clarify the localization of the water-soluble compounds, two kinds of autoradiographic procedures are available, i.e., autoradiography of soluble compounds and *in vitro* autoradiography using unfixed cryostat sections.

Nagata *et al.*, [*6*] started the autoradiography of water soluble or diffusible compounds in 1969. This method is the

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combination of administration of radiolabelled compounds, freeze-drying of the tissue, fixation with osmium vapor, direct Epon embedding under low temperature, tissue sectioning with ethylene glycol instead of water and application of autoradiographic emulsion film by wire-loop method. After 30 to 60-day exposure, the specimens were developed and fixed. Using this method, we could identify the uptake or binding sites of the radiolabelled chemicals by light and electron microscopy [*7*, *8*].

The second method is composed of cryostat sectioning of the unfixed tissues, administration of the radiolabelled compounds and application of autoradiographic emulsion films by the wire-loop method. In this method, the specimens can be observed by light microscopy and double staining with immunohistochemical method is available [*9*].

In this context, omeprazole and lansoprazole are very useful tools to clarify their uptake sites, because they are accumulated in the acidic compartment and binds covalently to the cysteine residues as mentioned above and become insoluble to water. Thus, the ordinary autoradiographic method can be applied which is used in ^{3}H-thymidine autoradiography, because administered ^{3}H-thymidine also becomes insoluble to water after incorporated to the DNA.

The specificity of the uptake sites of omeprazole and lansoprazole could be estimated by the prior administration of glutathione. This procedure is able to inhibit the accumulation of the compounds [*10*].

Uptake Sites in the Fundic Mucosa

In the healthy fundic mucosa, most of the uptake sites are the parietal cells (Fig. 1). The relatively young parietal cells localized in the neck portion of the funded glands show the strongest accumulation, while in the body and base of the funded glands the accumulation was not so strong. In addition, some of the uptake sites are recognized in the enterochromaffinlike cell and other neuroendocrine cells. These uptake sites could represent the same kind of P-type ATPases as reported to exist in the chromaffin cells and cholinergic nerves [*11*].

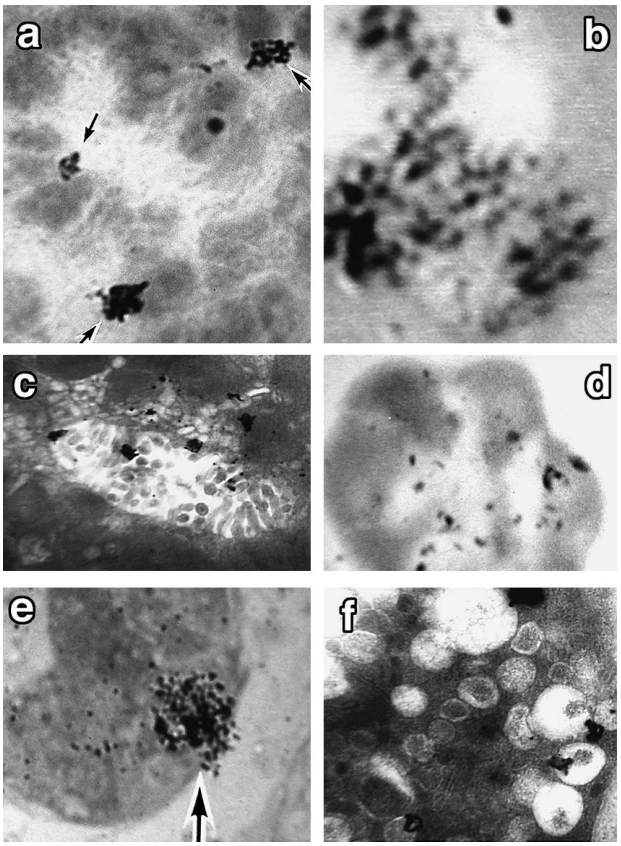

Fig. 1. Lansoprazole uptake sites in rat fundic mucosa and isolated parietal cells. Autoradiography of soluble compounds. a, b: Uptake sites are accumulated in the parietal cells. c: Electron microscopic autoradiograph showing that the silver grains showing the uptake sites are accumulated near the intracellular canaliculi of the parietal cell. d: In the isolated parietal cells, silver grains are recognized in the intracellular canaliculi. e, f: In the base of the fundic glands, silver grains are accumulated in the enterochromaffin-like cells.

Uptake Sites in Helicobacter pylori-Infected Mucosa

The uptake sites of PPIs in the *Helicobacter pylori* (*H. pylori*)-infected gastric mucosa can be divided into *H. pylori* itself and background mucosal tissues.

As to the activity of substituted benzimdazoles against *H. pylori*, Megraud *et al.* [*12*] reported the bacteriostatic effect of lansoprazole and omeprazole but not against *C. jejuni* or *E. coli*. The MICs was lower for lansoprazole than for omeprazole (16 vs 64 mg/l). Following this paper, members of the Takeda pharmaceutical have reported many interesting papers. At first they showed the inhibition of *H. pylori* urease activity by lansoprazole [*13*, *14*], while in the later papers they found the activity of lansoprazole was fourfold more potent than that of omeprazole and bismuth subsalicylate, with MICs ranging from 1.56 to 25 micrograms/ml and concluded that anti-*H. pylori* effect is not by sulfenamide, because the antibacterial activity of omeprazole and lansoprazole was not affected by glutathione or dithiothreitol, which completely abolished the inhibitory activity of lansoprazole against *H. pylori* urease [*15*, *16*].

As to the effect of other substituted benzimidazoles, omeprazole and rabeprazole to *H. pylori*, both of the urease dependent and independent mechanisms have been postulated [*17–19*]. As to this non-sulfenamide-mediated anti-*H. pylori* effect of proton pump inhibitors, the sulfides of benzimidazoles was reported to show the selective and reversible antibacterial effect without any covalent protein

binding [*20*].

As to the urease activity of *H. pylori*, not only the surface bound urease but UreI existing between two layers of bacterial membrane has also reported to play more significant role in the survival of *H. pylori*, and this enzyme system was suggested to be a new target of drug therapy [*21*].

As to the pharmacological foci of *H. pylori* other than urease, the existence of P-type ATPases has been suggested [*22–24*]. One of these enzymes was shown to be heavy metal cation, cupper and nickel, transporting ATPase and belongs to a family of P-type ATPases containing eight transmembrane segments. This enzyme was reported to have relation to bacterial resistance to heavy metals [*25*] and exists also in *Streptomyces* species [*26*]. More recently, this enzyme has shown to be related to bacterial adaptation to the environment and type IV-related secretory mechanism of Cag A [*27–30*].

As to the formation of the autoimmune gastritis, the molecular mimicry of *H. pylori* and the parietal cells have been pointed out. Recently, this is found to be related to the H, K-ATPase in the parietal cells [*31*, *32*].

Other ATPase system reported in *H. pylori* is the flagellar-specific ATPase (Fli1) [*33*] and V-type ATPase related to vac A [*34*].

Our autoradiographic study has shown the existence of uptake sites of ^{3}H-lansprazole near the plasma membrane of the *H. pylori* (Fig. 2) [*35*]. This localization could be related one of these enzymes described above.

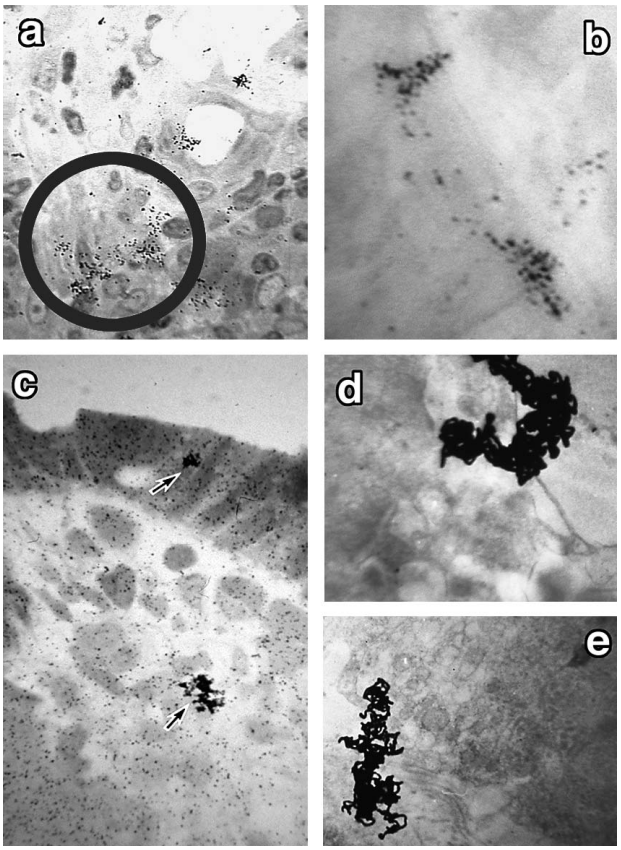

Fig. 2. Lansoprazole uptake sites in *H. pylori*-infected gastric mucosa. a, b: In *H. pylori*-infected Mongolian gerbil fundic mucosa (a), the uptake sites of lansoprazole are seen in the inflammatory cells in the lamina propria mucosa as well as parietal cells and *H. pylori*, while in the control gerbils the uptake sites are accumulated in the luminal side of the parietal cells. Autoradiography of soluble compounds. c: By *in vitro* autoradiography using unfixed cryostat sections of the Mongolian gerbil fundic mucosa, the uptake sites are seen near the surface epithelial cells and inflammatory cells (arrows). d, e: By Electron microscopic autoradiography of soluble compounds, the uptake sites are accumulated in the *H. pylori* (d) as well as inflammatory cells (e).

Uptake Sites in the Colonic Mucosa

There are sites other than fundic mucosa in the body to be able to reabsorb K and secrete H, such as the distal colon and the distal tubule of the kidney.

Kaunitz *et al.* [*36*] reported the existence of two kinds of colonic ATPases by the pharmacological method. One was similar to H, K-ATPase, while the colonic transporter in the intact organ was ouabain inhibitable, in contrast to the H, K-ATPase [*37*]. The sequence of the colonic APTase is 75% homologous to the H, K-ATPase in the parietal cell [*38*]. A K-ATPase has been shown to be present in a colon cancer cell line and be related to drug resistance [*39*, *40*]. It would appear that there is yet another family of P-type ATPases, those with sequences intermediate between the Na, K- and H, K-ATPases and suggested to have different inhibition characteristics [*41*]. The cloned colonic ATPase does not have the extracytoplasmic cisterns reacting with omeprazole, and the loop between M1 and M2 is not identical with the gastric H, K-ATPases.

Through our autoradiographic studies using ^{3}H-lansoprazole, we have obtained two diverse uptake sites in the colonic mucosa. By the *in vivo* administration followed by auto-radiography of soluble compounds, the uptake sites were mostly found in the upper colonic epithelial cells in the control rats (Fig. 3) [*8*], while in the *in vitro* autoradiography using unfixed cryostat sections, most of the uptake sites were found in the inflammatory cells including polymorphonuclear leucocytes and macrophages and in the colonic epithelial cells in the control and dextran sulfate sodium (DSS)-treated rats [*9*]. Because of the different method of fixation, this may be due to the stability of the enzyme in the inflammatory cells. The clinical relevance to this observation is not clear, but some reports showed the effectiveness of omeprazole to the ulcerative colitis [*42*] and DSS-induced colitis [*9*].

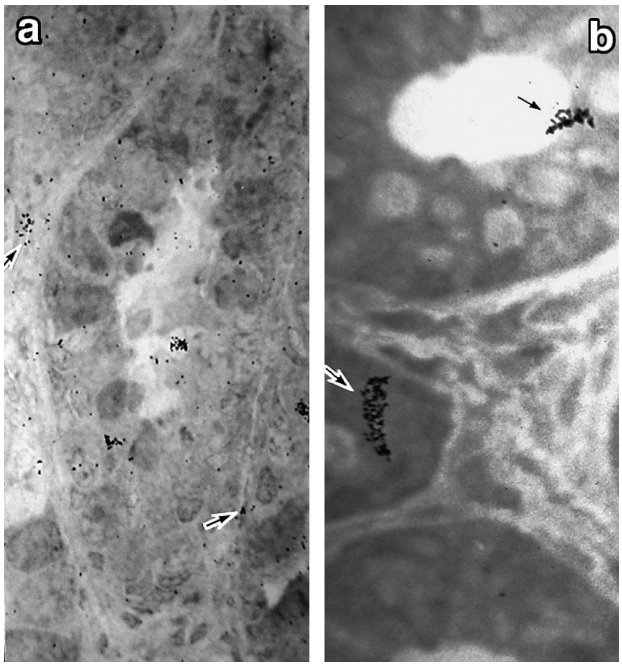

Fig. 3. Lansoprazole uptake sites in control colonic mucosa. a: By *in vitro* autoradiography of the control rat colonic mucosa, the uptake sites of the lansoprazole are seen in the inflammatory cells (arrows) as well as in the epithelial cells. b: By autoradiography of soluble compounds, the uptake sites are mostly seen in the luminal surface of the colonic epithelial cells (arrows).

Uptake in Inflammatory Cells

From the above observation, the relation of lansoprazole to the inflammatory cells has been suggested, while few reports have shown the existence of P-type ATPase in the inflammatory cells and V-type ATPase was proved to be existing through the effect of bafilomycin [*43*]. The pH of lysosome is generally thought to be about 5 and can be a candidate for the accumulation of substituted benzimidazole, especially lansoprazole, because it has two pKas and one is slightly shifted to the neutral pH, compared with omeprazole [*44*].

Relation to Other Bacteria

The benzimdazoles are found to be bacteriocidal to oral bacteria, *Streptococcus mutans*, *Fusobacterium nucleatum* and *Prevotella intermedia* in acidic environment [*45*]. In these bacteria, identified targets for benzimidazole inhibition included the phosphoenolpyruvate sugar phosphotransferase system, the glycolytic enzymes aldolase, glyceraldehyde-3-phosphate dehydrogenase, and lactic dehydrogenase, and enzymes such as urease and arginine deaminase.

Uptake in Fungus and Yeast

The yeast plasma membrane proton pumping ATPase (H-ATPase) is a potential molecular target for antifungal drug therapy by examining the inhibitory effects of the sulfhydryl-reactive reagent omeprazole on cell growth, glucose-induced medium acidification and H-ATPase activity [*46*]. Omeprazole inhibited the growth of *Saccharomyces cerevisiae* and the human pathogenic yeast *Candida albicans* in a pH dependent manner.

Uptake Sites in the Esophagus

We have observed the uptake sites of lansoprazole in the esophagus and found mostly in the peripheral autonomic nervous system. By the immunohistochemical observation, most of them coincided with CGRP-immunoreactive nerves.

Pharmacological studies have demonstrated the effect of lansoprazole on the increased bicarbonate secretion [*47*] and autonomic nerve-mediated regulation was suggested. As to the autonomic nerves, existence of P-type ATPase has been reported in the cholinergic and adrenergic nerves [*11*, *48*] and this is a functional protein system containing a critical sulfhydryl group.

Possible Uptake in Kidney, Placenta and Others

In rat kidney, sequence information has shown the presence of gene products identical with the gastric H, K-ATPase [*49*, *50*]. There has also been biochemical evidence described showing the presence of a K-ATPase in the kidney that is different from the Na, K-ATPase. In placenta, non-gastric H, K-ATPase is present in the microvillous plasma membrane of the transporting epithelia of the human placenta [*51*]. In addition, fibroblasts [*52*] and endothelial cells [*53*] could be possible candidates.

Conclusions

The effectiveness of substituted benzimidazoles seems to be clinically obvious, but we still have a long way to go to draw the whole map of this interesting agent especially its relation to various kinds of ATPases.

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