

## Changes in gastric ECL cells and parietal cells after long-term administration of high-dose omeprazole to patients with Barrett's esophagus

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(Received March 9, 2005; Accepted March 31, 2005)

**[Purpose]** Long-term administration of PPI causes hyperplastic changes of the gastric parietal cells; however, the detailed mechanism remains to be clarified. We administered high-dose omeprazole to patients with Barrett's esophagus for 2 years, and investigated changes in gastric ECL (Enterochromaffin-like) cells using endoscopic biopsy specimens to clarify the etiology of hyperplasia of the parietal cells.

**[Methods]** The subjects were 69 patients who were diagnosed as having Barrett's esophagus (39 males, 30 females). We established two groups, an omeprazole-treated group and a ranitidine-treated group. Upper digestive tract endoscopy was performed before administration, and 12 and 24 months after the start of administration. Biopsy was performed in the greater curvature of the gastric body. The ECL/parietal cell counts and the grade of hyperplasia of the gastric mucosa were determined under a microscope. In addition, the fasting serum gastrin level was measured, and statistical analysis was performed.

**[Results]** In the omeprazole-treated group, the ECL cell count was markedly increased 12 months after the start of administration, but was lower than the pretreatment value 24 months after the start of administration. The parietal and ECL cell counts significantly increased. Furthermore, there were no changes in mucosa thickness. The fasting serum gastrin level significantly increased.

In the ranitidine-treated group, there was no increase in the ECL cell count, and the parietal cell count was decreased. There was no significant increase in mucosa thickness. The fasting serum gastrin level increased, although the rate of increase was markedly smaller than that in the omeprazole-treated group.

**[Conclusion]** Not the direct pharmacological actions of PPI but hypergastrinemia-associated secondary changes may be etiologically involved in hyperplasia of the parietal cells related to long-term administration of PPI.

**Key words:** Enterochromaffin-like cell, omeprazole, ranitidine, Barrett's esophagus, hypergastrinemia, parietal cell

### INTRODUCTION

Hyperplastic changes of the gastric parietal cells are observed in patients treated with a proton pump inhibitor (PPI) for a long period. Concerning the mechanism, there are two hypotheses; hypergastrinemia or the direct actions of PPI may be involved. However, few studies have reported the detailed mechanism. If hypergastrinemia

is an etiological factor, the number of ECL cells, which produce histamine promoting the parietal cells, may be increased.

In this study, we administered omeprazole or a control agent, ranitidine, to patients with Barrett's esophagus. Endoscopy was performed before administration and 12 and 24 months after the start of administration. Using biopsy specimens collected from the greater curvature of the gastric body,

specific staining was performed, and the ECL cell and parietal cell count and the grade of hyperplasia of the gastric mucosa were determined under a microscope. In addition, the fasting serum gastrin level was measured, and statistical analysis was performed.

## SUBJECTS AND METHODS

### Subjects

The subjects were 69 patients in whom upper digestive tract endoscopy suggested Barrett's esophagus (39 males, 30 females). In selecting agents, these patients were randomly assigned to receive omeprazole or a control agent, ranitidine. The doses of omeprazole were 80 mg/day during the first year and 40 mg/day during the second year. The dose of ranitidine was 600 mg/day for 2 years. Upper digestive tract endoscopy was performed at 12-month intervals, and the greater curvature of the gastric body was biopsied using a forceps. Simultaneously, the fasting serum gastrin level was measured.

### Fasting serum gastrin

Blood (5 ml) was collected, and immediately centrifuged to isolate sera. Serum samples were frozen at  $-20^{\circ}\text{C}$ , and the fasting serum gastrin level was measured by radioimmunoassay.

### Histopathological investigation

In biopsy specimens, hematoxylin and eosin (HE) staining and Grimelius silver staining were performed. The ECL and parietal cells were counted. An ECL cell in which the nucleus could be recognized under a microscope was counted as a cell.

### Measurement of mucosa thickness

Using an eyepiece grid, the maximal distance between the mucosal surface and the mucosal myotome in biopsy specimens was measured as mucosa thickness.

### Statistical analysis

The data are expressed as the mean  $\pm$  standard deviation. Statistical analysis was performed using Mann-Whitney's method for unpaired non-parametric data. For the analysis of correlation, Spearman's rank test was used.  $P < 0.05$  was regarded as significant.

## RESULTS

### Fasting serum gastrin

In the omeprazole-treated group, the fasting serum gastrin level 12 months after the start of administration was about two times higher than the pretreatment value; however, 24 months after the start of administration, when the dose was decreased by half, it was about 1.5 times higher than the pretreatment value. There were significant differences. In the ranitidine-treated group, the fasting serum gastrin level was increased 12 and 24 months after the start of administration. However, the rate of increase was markedly smaller than that in the omeprazole-treated group, and there were no significant differences.

### Mucosa thickness

In the omeprazole-treated group, mucosa thickness 12 and 24 months after the start of administration was slightly higher than the pretreatment value; however, there were no significant differences. In the ranitidine-treated group, there were no significant increases 12 or 24 months after the start of administration.

### ECL cell count

In the omeprazole-treated group, the ECL cell count 12 months after the start of administration was significantly higher than the pretreatment value; however, 24 months after the start of administration, it was lower than the pretreatment value, although there was no significant difference. In the ranitidine-treated group, there were no increases in the ECL cell count 12 or 24 months after the start of administration.

### Parietal cell count

In the omeprazole-treated group, the parietal cell count 12 months after the start of administration was about two times higher than the pretreatment value, with a significant difference; however, 24 months after the start of administration, it was 1.5 times increased. In the ranitidine-treated group, the parietal cell count serially decreased compared to the pretreatment value, although there were no significant differences (Tables 1 and 2).

**Table 1** Changes of gastrin, thickness of mucosa, number of ECL cell and Parietal cell

	0 Month	12 Months	24 Months
Gastrin (ug/ml)	88.21+/-54.74	162.26+/-86.80*	136.44+/-80.06*
Thickness of mucosa(mm)	57.59+/-12.40	63.89+/-10.88	60.08+/-12.58
Total number of ECL cell	4.00+/-2.63	7.00+/-4.73*	2.58+/-1.64
Total number of parietal cell	71.53+/-19.91	114.33+/-23.79*	98.14+/-28.63*

Means and Standard Deviations      \* P < 0.05

**Table 2** Changes of gastrin, thickness of mucosa, number of ECL cell and Parietal cell in ranitidine group

	0 month	12 months	24 months
Gastrin (ug/ml)	69.36+/-21.33	87.54+/-28.25	85.16+/-39.54
Thickness of mucosa(mm)	54.11+/-10.94	54.77+/-9.66	52.50+/-8.30
Total number of ECL Cell	2.75+/-2.07	2.39+/-2.39	2.17+/-2.36
Total number of parietal Cell	74.00+/-22.94	66.44+/-15.52	59.44+/-12.42

Means and Standard Deviations      \* P < 0.05

## DISCUSSION

In humans, gastric endocrine cells comprise 0.5 to 1% of the fundic gland mucosa; among them, the main cells involved in the acid secretion mechanism include enterochromaffin (EC) cells, ECL cells, and G/D cells responsible for production of gastrin and somatostatin, and at least 7 kinds of endocrine cells have been reported in the stomach [1].

In 1981, Fellenius *et al.* [2] first reported a PPI, omeprazole. This agent is absorbed in the small intestine, and when it reaches the gastric mucosa, it potently inhibits gastric acid secretion by specifically inhibiting  $H^+/K^+$ -ATPase in the parietal cells, that is, gastric acid-secreting cells. Therefore, PPIs are commonly administered to treat gastroduodenal ulcers and Zollinger-Ellison syndrome, in which the basic condition involves the enhancement of acid secretion. Its

efficacy has been reported.

PPIs act on a proton pump ( $H^+/K^+$ -ATPase) in the final process of the extracellular release of acid ( $H^+$ ) in the parietal cells, potently inhibiting its activity, and suppressing acid secretion. Thus, a persistent increase in intragastric pH and a disorder in the acid-mediated physiological negative feedback mechanism of antral mucosa G cells promote production/release of gastrin in G cells, increasing the blood concentration of gastrin which promotes the function of ECL cells [3].

There is a close relationship between PPIs and digestive tract hormones. In particular, an animal experiment using rats has demonstrated that long-term administration of a PPI resulted in proliferation of gastric ECL cells, that is, the development of carcinoid tumors. In humans, PPI administration may also cause carcinoid tumors of the stomach; however, there has been no case report. The development of these tumors was observed

only in female rats with a high ECL cell count. In addition, many investigators have indicated that the development of carcinoid tumors of the stomach is related to a secondary effect via hypergastrinemia, not to the direct tumor-inducing feature of a PPI [4-6].

In humans, the incidence of carcinoid tumors of the stomach is low even in the presence of hypergastrinemia for the following two reasons: 1) the ECL cell/D cell ratios in the stomach are 2:1 in humans and 4:1 in rats, and the ECL cell count is relatively higher in rats; and 2) the number of D cells, which inhibit gastrin secretion via paracrine, is smaller in rats. Furthermore, in humans, mast cell-derived histamine distributed in the inherent mucosal layer, rather than ECL cell-derived histamine, controls parietal cell function, and the role of ECL cells is less significant with respect to production of histamine, which also contributes to the above finding.

Most carcinoid tumors of the stomach may be derived from ECL cells; a large number of ECL cells are distributed in the fundic gland region in many vertebrates, comprise approximately one-third of the gastric mucosal endocrine cells, and secrete not only histamine, which is directly involved in acid secretion, but also various hormones. However, the cell distribution differs among species [4].

Another limitation of omeprazole administered for a long period is proliferation of gastric endocrine cells [6-10].

Lamberts *et al.* [11] reported that long-term administration of 40 mg/day of omeprazole to humans (mean period: 4 years) increased the ECL cell count, the incidence of micronodular hyperplasia, and the serum gastrin level (2-fold).

Solcia *et al.* [12] also investigated morphological changes in the gastric mucosa related to long-term administration of omeprazole to humans. They administered 40 mg/day of omeprazole to 122 patients with H<sub>2</sub> blocker-resistant ulcers or reflux esophagitis in the acute stage, and subsequently administered 20 mg/day of omeprazole in the remission stage (mean total period: 13 months). In 43 patients (35.2%), some type of hyperplasia was observed; however, no patient developed heteroplasia or any carcinoid tumor of the stomach.

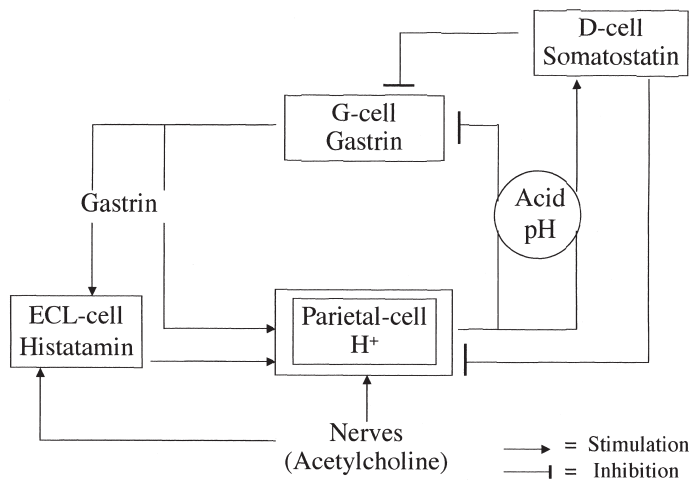
When we reviewed changes in the

serum gastrin level after administration of omeprazole or lansoprazole to humans [13], short-term administration of a PPI (2 to 3 months) increased the blood gastrin level 2 to 4 times the normal value; however, this value was markedly lower than that in patients with gastrinoma or malignant anemia (type A gastritis). In addition, it is known that the serum gastrin level 3 or more months after the start of administration of a routine dose of a PPI shows a plateau, and that it is rapidly reduced and normalized after discontinuation. Lind *et al.* [14] monitored changes in the serum gastrin level for 24 hours after administration of omeprazole or after selective proximal vagotomy (SPV) in humans. There was no significant difference in the changes in the plasma gastrin level between 8 patients with duodenal ulcers after SPV and 8 patients with duodenal ulcers after 4-week administration of omeprazole (20 mg/day), and the gastrin level was increased only 2- to 3-fold the normal value. The rate of increase in the gastrin level after administration of a PPI is smaller than that in patients with gastrinoma or malignant anemia (type A gastritis).

Stolte *et al.* [15] reported that a PPI, lansoprazole, induced hyperplasia of the parietal cells, and that discontinuation made this change reversible, as speculated from the same action mechanism as omeprazole.

In this study, we administered 80 mg/day of omeprazole for the first year and 40 mg/day of omeprazole for the second year, although the standard dose of omeprazole 40 mg/day for gastric ulcer in US. As a control agent, we administered 600 mg/day of ranitidine for 2 years despite a routine dose of 300 mg/day.

PPIs act on a proton pump in the final process of the extracellular release of acid in the parietal cells, potently inhibiting its activity, and suppressing acid secretion. Thus, a persistent increase in intragastric pH and a disorder in the acid-mediated physiological negative feedback mechanism of antral mucosa G cells promote production/release of gastrin in G cells, causing hypergastrinemia. Hypergastrinemia promotes ECL cells, and increases the ECL cell count and histamine release, thus causing hyperplasia of the parietal cells. The cause of decreasing ECL cell counts at 24 months in omeprazole group should be rebound phenomenon (Fig. 1).



**Fig. 1** Scheme of the stimulating and inhibitory pathways regulating gastrin and acid secretion

These results suggested that not the direct actions of a PPI on the parietal cells, but hypergastrinemia in a physiological route responsible for intragastric pH control was etiologically involved in hyperplasia of the gastric parietal cells in patients with Barrett's esophagus who had been treated with a high-dose PPI for 2 years.

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