Fine Structural and Morphometric Studies on Gastric Parietal Cells of Peptic Ulcer Patients after Long-Term Treatment with Omeprazole

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Summary. Omeprazole, a substituted benzimidazole, is known to inhibit acid secretion from parietal cells in gastric glands, and is widely utilized as a drug for peptic ulcer. To clarify the ultrastructural changes in parietal cells from long-term treatment with a therapeutic dose of omeprazole, biopsy specimens of the gastric mucosa obtained from peptic ulcer patients were morphometrically analyzed before and after omeprazole treatment. Before treatment with omeprazole, parietal cells in both the stimulated and resting stages were observed; the stimulated cells possessed smaller amounts of tubulovesicles in the cytoplasm and numerous profiles of microvilli in the intracellular canaliculi, whereas the cells in the resting phase showed numerous profiles of tubulovesicles and poorly developed microvilli in the canaliculi. Eight weeks after the onset of omeprazole treatment, the amounts of both tubulovesicles in the cytoplasm and microvilli in the intracellular canaliculi drastically decreased. These decreases in the profiles of the membrane structures with a proton pump occurred concomitantly with a significant increase in autophagic vacuole/autolysosome-like structures. These results suggest that the membrane structures with proton pump are not recycled between tubulovesicles and microvilli of intracellular canaliculi in parietal cells after omeprazole treatment, but may be sequestrated into autophagosomes and degraded by lysosomal enzymes.

Parietal cells in gastric mucosa secrete hydrochloric acid into the lumen. To generate gastric acid, a specific H⁺-K⁺-ATPase is expressed in parietal cells and acts as a proton pump. Gastric H⁺-K⁺-ATPase is localized on both the surfaces of intracellular canaliculi and tubulovesicles in the cytoplasm of the cells. When the parietal cells are stimulated, tubulovesicles are fused to the membrane of intracellular canaliculi, and H⁺-K⁺-ATPase on the membrane is translocated to the cell surface, where it exchanges hydrogen for potassium ions (Forste et al., 1981, 1989).

Omeprazole, a derivative of benzimidazoles, is known to inhibit gastric acid secretion by blocking H⁺-K⁺-ATPase activity (Fellenius et al., 1981; Clissold and Campoli-Richards, 1986). This substituted benzimidazole is widely used for diseases caused by excessive gastric acid, such as gastric ulcer, duodenal ulcer, reflux esophagitis, and Zollinger-Ellison syndrome. Omeprazole specifically binds H⁺-K⁺-ATPase molecules on the surface of parietal cells, and suppresses H⁺-K⁺-ATPase activity. However, the fate of H⁺-K⁺-ATPase molecules blocked by omeprazole is not fully elucidated.

It is well known that parietal cells structurally differ greatly between stimulated and non-stimulated resting phases (Forste et al., 1977, 1981, 1989; Helander 1981). When stimulated, the cells possess smaller amounts of tubulovesicles in the cytoplasm and numerous profiles of microvilli in the intracellular canaliculi, whereas in the resting phase the profiles of tubulovesicles increase and those of microvilli decrease. We have previously examined the effects of omeprazole on these subcellular structures of parietal cells in the rat gastric gland, using morphometric techniques (Furushashi et al., 1992). After administration of omeprazole to rats, the volume densities of both tubulovesicles and canalicular microvilli, where the proton pump is located, significantly decrease, while that of lysosomal structures increases. These findings suggest that H⁺-K⁺-ATPase inactivated by omeprazole is degraded in the autophagosome/autolysosome of the cells.
To further understand the fate of the membranes with H⁺-K⁺-ATPase in parietal cells obtained from patients suffering from peptic ulcer and treated with a therapeutic dose of omeprazole, we analyzed the ultrastructural changes in gastric parietal cells of biopsy specimens using morphometric methods. The present data demonstrated that omeprazole decreased the amounts of tubulovesicles and microvilli in the intracellular canaliculi of the parietal cells and increased that of lysosomal compartments, suggesting that membrane structures with a proton pump inactivated by omeprazole are not recycled between tubulovesicles and intracellular canaliculi, but may be degraded in lysosomal compartments of gastric parietal cells. In the present study, we discuss the possibility that the suppressive effects of omeprazole on the gastric acid secretion are enhanced by the degradation of the proton pump inhibited by omeprazole.

MATERIALS AND METHODS

Experimental subject

As summarized in Table 1, four patients suffering from gastric ulcer or duodenal ulcer comprised this study. Each patient was treated daily with 20 mg omeprazole (Yoshitomi Pharmaceutical Co., or Fujiwara Pharmaceutical Co., Japan) for 8 weeks. Endoscopy was performed before and after treatment to determine the clinical stage of each ulcer. At the same time, biopsy specimens were obtained from the midpart of the gastric body mucosae along the greater curvature. Informed consent for all procedures was obtained from all patients. Culture and urease tests of biopsy specimens were additionally performed to examine Helicobacter pylori infection.

Electron microscopy

For electron microscopy, biopsy specimens of gastric mucosa (n=4 per each patient) were immediately fixed in a mixture of 2% glutaraldehyde-2% paraformaldehyde buffered with 0.1 M phosphate buffer, pH 7.2, and immersed in the same fixative at 4°C overnight. The samples were then postfixed in 1% OsO₄ buffered with 0.1 M phosphate buffer containing 7.5% sucrose (pH 7.2), for 2 h. After dehydration with graded series of ethanol, they were embedded in Epon 812.

Silver sections were cut with an ultramicrotome, and contrasted with saturated aqueous solutions of uranyl acetate and lead citrate. Stained sections were observed with a Hitachi H-7000 electron microscope, and photographed at original magnifications of ×1,800, calibrated using a carbon grating replica with 2,000 lines per mm. Six fields of parietal cells were chosen randomly from each specimen, and four specimens were analyzed for each case; twenty-four fields of parietal cells from each patient were photographed for the following morphometric analyses.

Morphometric analyses

The morphometric method was performed as previously reported (FURUHASHI et al., 1992). Briefly, the point-counting method by WEIBEL (1979) was applied to measurements of the volume, surface and numerical profile densities (Vv, Sv and NA) of various subcellular structures, including tubulovesicles, intracellular canaliculi, canalicular microvilli, multivesicular bodies, autophagosomes, mitochondria, rough endoplasmic reticulum, Golgi apparatus, and ground cytoplasm in parietal cells. The volume densities (Vv) of various cytoplasmic organelles and their numerical profile densities (NA) were analyzed at 10 times the original magnification by a universal projector, using a double-lattice system with 1.5 cm-spacing (test points: 234). For estimation of the surface densities, the number of intersection points of cytoplasmic membranes with the test line was counted on the same screen of the instrument at the same magnification.

Twenty-four micrographs taken from each patient before and after omeprazole treatment were respectively analyzed. The values of the morphometric parameters for each subcellular structure were calculated from each electron micrograph, and the mean, standard deviation, and standard error of the mean (SE) of the values obtained from each photograph were calculated for each morphometric parameter. The mean values calculated were statistically

Table 1. Clinical summary of the patients studied

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>HP infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>F</td>
<td>Gastric ulcer</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>F</td>
<td>Duodenal ulcer</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>M</td>
<td>Gastric ulcer</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>M</td>
<td>Gastric ulcer</td>
<td>+</td>
</tr>
</tbody>
</table>

M: male, F: female, HP: Helicobacter pylori
Fig. 1. Parietal cells in the gastric glands obtained from a patient with a gastric ulcer before treatment with omeprazole. They possess numerous mitochondria (M) with well developed cristae and tubulovesicles (TV) surrounding intracellular canaliculi (IC, a). Microvilli on intracellular canaliculi are fewer and shorter in height in the parietal cells, whereas numerous profiles of tubulovesicles are visible near the canaliculi (b). Parietal cells with well-developed microvilli on intracellular canaliculi are also observed, but the profiles of tubulovesicles in these cells appear less in number (c). N nucleus. a: ×9,000, b and c: ×21,000
Fig. 2. Parietal cells in the gastric glands obtained from the same patient as in Figure 1 after treatment with omeprazole for 8 weeks. Tubulovesicular structures decrease in the cytoplasm, and intracellular canaliculi (IC) with shortened microvilli appear less frequently (a). Autophagic vacuole-like structures (AP) containing the remnants of membranous structures are seen in the cytoplasm near the canaliculi (IC, b). (Continued on p. 291)
Multivesicular body-like (MV) or autophagic vacuole-like (AP) structures also appear in the cytoplasm (c). Dense bodies (DB) containing lamellar structures are also frequently observed in the cytoplasm (d). M mitochondria, N nuclei. a: x7,000, b: x16,000, c: x14,000, d: x18,000
compared between those obtained before and after treatment by analysis of variance (ANOVA) using Tukey's Honestly Significant Difference (HSD) test. Corrections of systematic errors occurring in the volume, surface and numerical densities of various components were performed according to WEIBEL (1979).

RESULTS

Electron microscopy

Parietal cells in the gastric glands obtained from non-treated patients were characterized by the presence of abundant mitochondria with well developed cristae and tubulovesicles surrounding intracellular canaliculi where microvilli were developed (Fig. 1a). In the parietal cells examined, when numerous profiles of tubulovesicles were present in the vicinity of intracellular canaliculi, microvilli on the canaliculi were fewer and shorter in height (Fig. 1b). On the contrary, when profiles of tubulovesicles decreased in number, the microvilli increased in both number and height (Fig. 1c).

Eight weeks after the onset of treatment with a therapeutic dose of omeprazole, tubulovesicular structures apparently decreased in the cytoplasm of parietal cells (Fig. 2a). Microvilli of intracellular canaliculi were shortened in height and decreased in number, while multivesicular body-like or autophagic vacuole-like structures containing the remnants of membranous structures often appeared in the cytoplasm near the canaliculi (Fig. 2b, c). Dense bodies containing lamellar structures also became prominent in parietal cells of the omeprazole-treated patients (Fig. 2d).

Morphometry

To evaluate quantitatively the changes in the ultrastructure of parietal cells after omeprazole treatment, morphometric parameters of various organelles were determined (Fig. 3). Outstanding alterations were discerned in the morphometric parameters of the membranous structures with a proton pump. That is, the volume and surface densities of tubulovesicles and canalicular microvilli markedly decreased 8 weeks after the onset of omeprazole treatment (Fig. 3a, b). The surface density of intracellular canaliculi also decreased significantly after omeprazole treatment, whereas no clear-cut difference was detected in the volume density (Fig. 3c).

Contrasting with the prominent decreases in the morphometric parameters of tubulovesicles and microvilli, the volume, surface, and numerical profile densities of both multivesicular body-like structures and autophagic vacuoles/autophagolysosomes increased drastically after omeprazole treatment (Fig. 3d). The morphometric parameters of mitochondria, which occupied large parts of the cytoplasm in the parietal cells, did not alter between those obtained before and after omeprazole treatment (Fig. 3f). The rough endoplasmic reticulum (rER) and Golgi complex related to synthesis and transport of H+·K+-ATPase were also morphometrically analyzed in parietal cells. The volume densities of the rER and Golgi complex were elevated after omeprazole treatment, although the change in the volume density of the rER was not significant (Fig. 3g, h).

DISCUSSION

Our present study using morphological and morphometric techniques demonstrated that membrane structures with a proton pump such as tubulovesicles and microvilli of intracellular canaliculi drastically decreased their amounts in parietal cells of patients with a peptic ulcer after long-term treatment with a therapeutic dosage of omeprazole. Moreover, these decreases in the amounts of membrane structures with a proton pump occurred concomitantly with an increase in the amount of autophagic vacuoles/autophagolysosomes in the cells. The data showing these alterations in the parietal cells of patients after omeprazole treatment are consistent with our previous results using rat parietal cells (FURUHASHI et al., 1992).

It is well known that omeprazole strongly suppresses acid production in gastric parietal cells (OLBE et al., 1979; FELLENIUS et al., 1981; WALLMARK et al., 1983; CLISSOLD and CAMPOLI-RICHARDS, 1986). The inhibitory effect of omeprazole on gastric acid secretion is attributed to the covalent binding to certain cysteine residues in the α-subunit of the H+·K+-ATPase molecule; this binding is essential for the suppression of proton pump activity (IM et al., 1985). In the physiological states of parietal cells, that is, both in the stimulated and resting states of normal parietal cells, the total amount of the proton pump in the parietal cell is believed to be relatively constant, although the intracellular localization of the proton pump alters depending on physiological conditions (FORTE et al., 1977, 1981, 1989; HELANDER, 1981). The proton pump is translocated to the surface of intracellular canaliculi in the stimulated cells, and
Fig. 3. Comparison of volume (Vv; $\mu m^3/\mu m^3$), surface (Sv; $\mu m^2/\mu m^3$), and numerical profile (NA; $1/\mu m^2$) densities of various subcellular structures in parietal cells of peptic ulcer patients between those before treatment (CONT) and 8 weeks after the onset of treatment (OMZ/8 wks) with omeprazole (Tukey's HSD test, *P<0.05, **P<0.01). Each graph shows the morphometric parameter(s) of tubulovesicles (a), microvilli (b), intracellular canaliculi (c), multivesicular bodies (d), autophagic vacuoles/autophagolysosomes (e), mitochondria (f), rough endoplasmic reticulum (g), and Golgi complex (h). Volume density: white column, surface density: column with transverse lines, numerical profile density: column with oblique lines, vertical bar: ±S.E.
retrieved from the cell surface to tubulovesicles in the cytoplasm of the resting cells. According to our present and previous studies (FURUHASHI et al., 1992), however, both tubulovesicles and canalicular microvilli drastically decreased in parietal cells after long-term treatment with omeprazole. This suggests that no recycling of membranes with a proton pump occurs between tubulovesicles and intracellular canaliculi.

There have been several reports to date concerning the effects of omeprazole on the ultrastructure of gastric parietal cells (STACHURA et al., 1983; KARASAWA et al., 1988; FURUHASHI et al., 1992; KARAM and FORTE, 1994; KATO et al., 1994; SCOTT et al., 1994). Studies on parietal cell responses to histamine stimulation after short-term pretreatments with omeprazole or histamine H₂-receptor antagonists such as ranitidine and cimetidine have clearly demonstrated that omeprazole and histamine H₂-receptor antagonists affect the ultrastructure of parietal cells in different ways, although both of them functionally suppress acid secretion from parietal cells (STACHURA et al., 1983; KARASAWA et al., 1988). Pretreatment with histamine H₂-receptor antagonists blocks the histamine-induced transition of parietal cells to an active state and induces a resting state for parietal cells. In contrast, pretreatment with omeprazole can not prevent the morphological transition to an active state; tubulovesicles in the cytoplasm of parietal cells are remarkably decreased by histamine stimulation, even if the animals are pretreated with omeprazole. These different ultrastructural alterations in parietal cells are likely due to the differences in the regulatory mechanisms between the two types of drugs. Omeprazole binds gastric H⁺-K⁺-ATPase, resulting in the direct inactivation of proton pump activity at the final step in the gastric acid secretion, whereas histamine H₂-receptor antagonists block the stimulating signal to the parietal cells at the receptor site located in the basal cell membrane.

More chronic effects of omeprazole treatment on the ultrastructure of parietal cells have also been examined by our group and others (KARASAWA et al., 1988; FURUHASHI et al., 1992; KARAM and FORTE, 1994; KATO et al., 1994; SCOTT et al., 1994). The present study clearly demonstrated that the drastic decreases in the amounts of tubulovesicles and microvilli of the intracellular canaliculi occurred concomitantly with a significant increase in the amount of lysosomal structures in parietal cells of peptic ulcer patients treated with a therapeutic dose of omeprazole for 8 weeks. Such an increase in the amount of lysosomal structures has also been shown in the parietal cells of rabbits treated with omeprazole for five days (KARAM and FORTE, 1994; SCOTT et al., 1994). In particular, SCOTT et al. (1994) analyzed morphometrically the number of lysosome-like structures per parietal cell profile in the rabbit, and found a six-fold increase after five days of omeprazole treatment. These lines of evidence suggest that the membrane structure with a proton pump, which is normally recycling between tubulovesicles and the surface of intracellular canaliculi, is degraded in autophagolysosomal compartments after prolonged treatment with omeprazole.

According to KARAM and FORTE (1994), the ultrastructure of parietal cells after 3 days of recovery from omeprazole is generally similar to that in control animals, although the formation of autophagic vacuoles by the omeprazole treatment is supposed to induce the degeneration of parietal cells. On the other hand, no serious ultrastructural change has been shown in mitochondria and nuclei, which may be indicative of cell degeneration, in parietal cells of peptic ulcer patients treated with a therapeutic dosage of omeprazole and/or histamine H₂-receptor antagonists (KATO et al., 1994). In any case, the ultrastructural changes in parietal cells from long-term omeprazole treatment seem to be recoverable after the cessation of omeprazole administration.

In summary, we analyzed fine structurally as well as morphometrically changes in gastric parietal cells of peptic ulcer patients before and after long-term treatment with a therapeutic dosage of omeprazole. The ultrastructure of parietal cells after omeprazole treatment differed from that of either resting or stimulated cells in the normal epithelium of the gastric gland; the amounts of both tubulovesicles and canalicular microvilli decreased, while those of multivesicular body-like structures and autophagic vacuoles/autophagolysosomes increased. These findings suggest that the membranes possessing H⁺-K⁺-ATPase inactivated by binding covalently to omeprazole are degraded in lysosomal compartments of parietal cells. The degradation of an inactivated proton pump in these compartments possibly enhances the suppressive effects of omeprazole on the gastric acid secretion. Further studies are required to clarify the fate of the gastric proton pump bound with omeprazole within the parietal cells.
REFERENCES


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