IMMUNOELECTRON MICROSCOPIC INVESTIGATION OF GLUTATHIONE-PEROXIDASE IN THE RAT STOMACH

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Immunocytochemical localization of glutathione-peroxidase (GSH-PO) in the stomach was studied in rats under the following experimental conditions; 1) normal untreated 2) histamine-administration 3) atropine-administration in order to clarify the role of GSH-PO. In normal untreated group, GSH-PO was mainly observed in the central part of the gland. At higher magnification, GSH-PO was diffusely distributed in the pyramidal or oval-shaped cytoplasm. Intracellular localization of GSH-PO was predominantly observed in the cytoplasm of the parietal cells. In histamine-administered animal, GSH-PO was intensely stained in cytoplasm of the parietal cells with well-developed secretory canaliculi. In atropine-administered animal, the intensity of the GSH-PO staining in the parietal cells was remarkably decreased. By immunoelectron microscopic investigations, GSH-PO was noted in cytoplasm as small granules or lysosome-like structures.

Based on our data, it is suggested that GSH-PO in the gastric parietal cells may play an important role in the prevention of damage to the microsomes with lipid peroxides induced during the process of HCl secretion. We further speculated that GSH-PO within the lysosome-like structures after atropine-administration might be a result of the degradation process of the peroxidized materials.

Oxygen-derived free radicals have been implicated from pharmacological studies with mucosal lesions induced by ischemia in the stomach. Recently, Thaete *et al.* (19) reported that immunohistochemical localization of superoxide dismutase, an enzyme that catalyzes the dismutation of the superoxide anion as a toxic free radical, was predominantly observed in the gastric parietal cells. On the other hand, it is a well documented fact that oxygen derived free radicals result in lipid peroxidation and damage of cellular membranes with the release of intracellular components, e.g., lysosomal enzymes, leading to further tissue damage (3, 6). It is well known that glutathione-peroxidase (GSH-PO) is a catalyse in the reduction of lipid peroxides. Therefore, it was anticipated that the GSH-PO in the stomach may play a role in reduction of lipid peroxides.

In the present study, in order to clarify the role of GSH-PO in the stomach, at-

tempts were made to localize the GSH-PO immunocytochemically in the stomach of normal, histamine- or atropine-administered rats.

MATERIALS AND METHODS

Animals and tissue preparation

Male Wistar rats, weighing 180 to 200 g, were fasted overnight. Group 1 consisted of untreated controls. In group 2, rats were injected subcutaneously with 0.5 mg/kg of histamine (Wako Pure Chemical Co.). In group 3, rats were injected subcutaneously with 0.5 mg/kg of atropine (Wako Pure Chemical Co.). In groups 2 and 3, rats were sacrificed 2 hr after drug administration. The rat was killed by decapitation and small pieces of the glandular stomach excised. The tissues were fixed in periodate-lysine-4% paraformaldehyde solution (11) for 18 to 20 hr at 4°C under constant agitation. The fixed tissues were then washed in 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 20% sucrose overnight at 4°C. Subsequently, 6 μ m frozen sections were prepared in a cryostat, and were mounted on albumin-coated glass slides. The sections were washed in 0.01 M PBS, pH 7.4, and then were stained by Nakane's direct peroxidase-labeled antibody method using anti-GSH-PO IgG Fab fragment (16, 22, 23).

For light microscopic observations of GSH-PO, 6 μ m frozen sections were incubated with the antibody labeled with horseradish peroxidase (HRPO, Sigma Co.) for 1 hr. After the incubation was completed, the sections were incubated in Graham-Karnovsky's reaction medium (7) which contained 3, 3'-diaminobenzidine (DAB, Wako Pure Chemical Co.) and 0.005% H_2O_2 as the substrate for 5 to 10 min. Then the sections were counterstained for nuclei with 1% methyl green dissolved in veronal acetate buffer, pH 4.2.

For electron microscopic observations of GSH-PO, 6 μ m frozen sections were incubated with HRPO labeled antibody for 6 hr. The sections were then incubated for 30 min in Graham-Karnovsky's reaction medium (7) from which the substrate, H_2O_2 , was omitted, and then incubated in the fully equipped reaction medium for 5 min. The sections were post-fixed in 2% OsO_4 in 0.1 M sodium phosphate buffer, pH 7.4, for 90 min, dehydrated in a graded ethanol series and embedded in Quetol 812 by the inverted gelatin capsule method. Ultrathin sections were prepared with LKB ultramicrotome and were observed in a JEOL 1200EX electron microscope.

As an immunologic negative control, normal rabbit serum (NRS) IgG Fab fragment labeled with HRPO was applied on both light and electron microscopic observations instead of anti-GSH-PO IgG Fab fragment labeled with HRPO.

RESULTS

In the rat glandular stomach, GSH-PO-positive cells were localized mainly in the central part of the gland (Fig. 1A). At higher magnification, GSH-PO was distributed diffusely in the pyramidal or oval-shaped cytoplasm (Fig. 1B). The control serum (NRS) was negative for immunohistochemical localization of GSH-PO in the rat stomach (Fig. 1C). By immunoelectron microscopic observations, GSH-PO was noted in cytoplasm of the parietal cells with "tubulovesicular system" or "tubulovesicles" and secretory canaliculi (Fig. 2).

In histamine-administered rat stomach, GSH-PO-positive cells were localized in the central part of the gland (Fig. 1D). This staining pattern of GSH-PO in the glandular stomach was similar to that of the control. By immunoelectron microscopic investigations, GSH-PO was predominantly localized in the cytoplasm of the parietal cells with well-developed secretory canaliculi (Fig. 3). The intensity of the GSH-PO staining near the well-developed secretory canaliculi was stronger than that of the controls.

In atropine-administered rat stomach, GSH-PO-positive cells were localized mainly in the central part of the gland (Fig. 1E). The intensity of the GSH-PO staining was remarkably decreased. By immunoelectron microscopic investigations, GSH-PO was noted in the cytoplasm of the parietal cells with tubulovesicular system. Furthermore, GSH-PO was seen in the cytoplasm as small granules or lysosome-like structure (Figs. 4, 5).

DISCUSSION

In the gastric parietal cells it has been suggested that the tubulovesicular system is the site of production of gastric acid which is subsequently released into the secretory canaliculi (9, 18). In fact, gastric acid secretion is accompanied by ultrastructural alterations in the parietal cells. Many researchers agree that the ultrastructural responses to stimulants such as histamine, gastrin or insulin include a proliferation of secretory canaliculi and a depletion of tubulovesicular system (1, 5, 18, 20). Thus, gastric acid-secreting and non-secreting parietal cells can be easily classified in the gastric gland by ultrastructural investigations.

In the present study, we found that immunohistochemical localization of GSH-PO in the rat stomach was detected in the parietal cells. The gastric parietal cell maintains a steep ionic gradient as it secretes HCl and must tolerate a high hydrogen ion concentration and ionic gradients are highly vulnerable to intracellular superoxide anion concentrations (2). Recently, Thaete *et al.* (19) reported that superoxide dismutase, an enzyme that catalyzes the dismutation of superoxide anion as a toxic free radical, was predominantly localized in the gastric parietal cells of the rats by immunohistochemical investigations. In addition, production of free radicals may initiate very rapid lipid peroxidation. It is generally accepted that lipid peroxidation is the reaction of oxidative deterioration of lipids and peroxidation involves the direct action of oxygen and lipid to form free radical intermediates and to produce semistable

FIG. 1A. Immunohistochemical localization of GSH-PO in control rat glandular stomach. GSH-PO is observed mainly in the central part of the gland. Peroxidase-labeled antibody method. $\times 100$

FIG. 1B. Higher magnification of Fig. 1A. GSH-PO is diffusely distributed in the pyramidal or oval shaped cytoplasm. Peroxidase-labeled antibody method. $\times 160$

FIG. 1C. Using control serum (Normal rabbit serum), no immunohistochemical reaction is seen in rat glandular stomach. Peroxidase-labeled antibody method. $\times 100$

FIG. 1D. Immunohistochemical localization of GSH-PO in histamine-administered rat glandular stomach. Peroxidase-labeled antibody method. $\times 100$

FIG. 1E. Immunohistochemical localization of GSH-PO in atropine-administered rat glandular stomach. The intensity of the GSH-PO staining is remarkably decreased. Peroxidase-labeled antibody method. $\times 100$

FIG. 2. Immunocytochemical localization of GSH-PO in the parietal cells of control rat stomach. GSH-PO is seen in cytoplasm. N: Nucleus. Bar= $1\text{ }\mu\text{m} \times 7,000$

FIG. 3. Immunocytochemical localization of GSH-PO in the parietal cells of histamine-administered rat stomach. Secretory canaliculi is well developed. GSH-PO is clearly detected in cytoplasm. Bar= $1\text{ }\mu\text{m} \times 12,000$

FIGS. 4, 5. Immunocytochemical localization of GSH-PO in the parietal cells of atropine-administered rat stomach. GSH-PO is seen as small granules or lysosome-like structures. N: Nucleus. Bars=1 μ m Fig. 4 $\times$ 7,000 Fig. 5 $\times$ 15,000

peroxides. Thus, free radicals result in the lipid peroxidation and damage of cellular membrane with release of intracellular components, e.g., lysosomal enzymes, leading to further tissue damage (3, 6). GSH-PO has been well recognized that it effectively reduces the lipid peroxides.

Therefore, GSH-PO in the gastric parietal cells might play an important role in prevention of damage with lipid peroxides induced during the process of HCl production and/or secretion.

By immunoelectron microscopic investigations, GSH-PO was predominantly localized in the cytoplasm of the gastric parietal cells. It is generally accepted that tubulovesicular system and secretory canaliculi is part of a system of membranes which moves rapidly between cytoplasm and surface on appropriate stimulation (4, 10). Thus, gastric parietal cells are rapidly transformed between acid-secreting and non-secreting on appropriate stimulation. In this respect, lipid peroxidation may occur in the microsomes including tubulovesicular system and secretory canaliculi. In fact, the microsomal membranes contain a relatively large amount of polyunsaturated fatty acid in their phospholipid and the microsomes are very liable to lipid peroxidation and concurrent damage (17, 21). Therefore, GSH-PO in the gastric parietal cells might play an important role in prevention of damage to the microsomes with lipid peroxidation. By histamine-administration, immunoelectron microscopically, GSH-PO was predominantly localized in the cytoplasm and the intensity of GSH-PO staining near the well-developed secretory canaliculi was stronger than that of the control. In contrast, by atropine-administration, the intensity of the GSH-PO staining was remarkably decreased. The findings may indicate that the lipid peroxidation rate is less in the non-secreting parietal cells after atropine-administration than in the acid-secreting parietal cells.

Based on our data and these facts, it is suggested that GSH-PO in the gastric parietal cells might play an important role in prevention of damage to membrane with lipid peroxides induced during the process of HCl secretion.

On the other hand, immunoelectron microscopic localization of GSH-PO in the parietal cells after atropine-administration was observed in the form of characteristic lysosome-like structures. Similar findings have been observed in the rat testicular- or peritoneal-macrophages and the glandular epithelial cells of the prostate by us (12-15). In this case, we postulated that lipid peroxidation may be continued even during the process of the degradation within the lysosome-like structure. Thus, GSH-PO-positive granules are thought to be a result of the degradation process of the peroxidized materials. Therefore, the results of the present study suggest that degradation of the microsomes in the parietal cells after atropine-administration may be increased. The true nature of GSH-PO in the gastric parietal cells after atropine-administration is to be further clarified.

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