An Electron Microscopic Study of the Pancreas and Parotid Gland of Rats with Experimental Acute Pancreatitis

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ISHIDATE, T., SENoo, A., KARIZAKI, G., SAITO, T., FUJIWARA, Y. and NIHEI, T. An Electron Microscopic Study of the Pancreas and Parotid Gland of Rats with Experimental Acute Pancreatitis. Tohoku J. exp. Med., 1974, 113 (3), 213-223 —— Electron microscopic observations of the parotid gland and the pancreas were done on rats with acute pancreatitis, obstructive jaundice, ileus or peritonitis produced as reported previously. In acute pancreatitis the ultrastructural changes noted in the parotid acinar cell were an irregular arrangement of the acinar cells associated with atrophy, decrease in number of secretory granules, dilatation of cisternae of the granular endoplasmic reticulum and appearance of lipid droplets. In other sets of experiments, however, these ultrastructural changes were not noted; the appearance of lipid droplets was the only finding in the obstructive jaundice produced by ligation of common bile duct at the hilar portion of liver. The ultrastructural features of the parotid acinar cells in the experimentally produced pancreatitis were compatible with histological findings previously reported. It is conceivable that the ultrastructural changes, except appearance of lipid droplets, in the parotid gland are induced by pancreatitis. The results of the present study provide additional evidence for the close relationship between pancreas and parotid gland. —— experimental pancreatitis; ultrastructure of pancreas; ultrastructure of parotid gland

In a series of our investigations concerning correlation between pancreas and parotid gland it has been shown that, in experimentally produced pancreatitis, the parotid gland became atrophic and degenerative and showed hypofunction (Kakizaki et al. 1971, 1972a, b, c, 1974). The present study describes the ultrastructural changes in the parotid gland and pancreas of rats with experimentally produced pancreatitis in comparison with those seen in other conditions such as perforative peritonitis, ileus and obstructive jaundice.

MATERIALS AND METHODS

Experimental animals used were 45 male rats of Wistar strain weighing 380-460 g. Five of these were used as untreated controls and the remaining 40 were divided into four groups. In each set of experiments the pancreas and parotid gland were removed for histological and electron microscopical studies.

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Production of acute pancreatitis (20 rats): Acute pancreatitis was produced in 20 rats after the method of Block et al. (1954). Laparotomy was performed under ether anesthesia and the common bile duct was ligated at the orifice into the duodenum. At 24, 48, 72 and 96 hours after ligation the pancreas and parotid gland were removed.

Production of obstructive jaundice (5 rats): The common bile duct was ligated at the liver hilar portion. At 96 hours following this operation, the pancreas and parotid gland were removed.

Production of perforative peritonitis (10 rats): Upon laparotomy the duodenum was transected completely at the site about 1 cm apart from the pylorus. At 24 and 96 hours following this operation, the pancreas and parotid gland were removed.

Production of intestinal obstruction (5 rats): The intestine was ligated at the site about 7.5 cm apart from the pylorus, and 24 hours later, the pancreas and parotid gland were removed.

The pancreas and parotid gland were treated with 3% glutaraldehyde solution (pH 7.3) after the method of Sabatini et al. (1963), at 4°C for 3 hours. After prefixation, the tissue was washed in several changes of isotonic phosphate buffer solution, and was postfixed for 2 hours in an ice-cold (4°C) solution of 1% OsO₄ buffered at pH 7.3 according to the method of Millonig (1961). The fixed material was dehydrated rapidly with ethanol series, and embedded in Epon 812 (Luft 1961). Before preparing ultrathin sections, 1 μ-thick sections were cut with glass knives and stained with toluidine blue for light microscopy. Double staining method with uranyl acetate and lead citrate was applied to the ultrathin sections on the grids. Sections were examined and photographed with a HU-12 or a JEM 100B electron microscope at an accelerating voltage of 75 or 80KV.

Results

Group of acute pancreatitis

24 hours after ligation: Slight decrease in number of secretory granules and mild dilatation of cisternae of the granular endoplasmic reticulum were noted in the pancreatic acinar cells. In the parotid gland, no remarkable histological and ultrastructural changes were seen.

48 hours after ligation: In the pancreas, slight dilatation of the central lumen of acini was noted sparsely. In addition to the decrease in number of secretory granules and the dilatation of granular endoplasmic reticulum, several round vacuoles which seemed to be transformed from elongated granular endoplasmic reticulum appeared in the cytoplasm of acinar cells.

In the parotid gland, both light and electron microscopies showed slight decrease of secretory granules in the acinal cells, but no other ultrastructural alterations of the cells.

72 hours after ligation: In the pancreas, histological changes similar to those seen in the non-experimental acute pancreatitis, such as interstitial edema, inflammatory cell infiltrations, dissociation and disarrangement of acinar cells, degranulation of acinar cells and dilatation of acinar lumen, were clearly observed. The ultrastructural observation revealed marked decrease of secretory granules and appearance of lipid droplets with moderate density, about 1 μ in diameter, in the
Fig. 1. Pancreas 72 hours after ligation of the common bile duct at the orifice to the duodenum. The acinar cell contains a small number of secretory granules (Sg), round vacuoles (V) and small vesicular granular endoplasmic reticulum (Er). Original magnification ×4,000.

Fig. 2. Parotid gland 72 hours after ligation of the common bile duct at the orifice to the duodenum. Acinar cells contain numerous secretory granules (Sg) of various electron density. Original magnification ×5,000.

C, central acinar cell; CT, connective tissue; Er, endoplasmic reticulum; G, Golgi apparatus; Li, lipid droplet; Lu, central lumen; M, mitochondria; N, nucleus; Sg, secretory granules; V, vacuoles; SC, secretory canaliculi.
basal and perinuclear portions of acinar cell. Dilatation of the granular endoplasmic reticulum was conspicuous in some acinar cells (Fig. 1). Thus, density of the cytoplasm varied to some extent giving the impression of “clear” and “dark” acinar cells.
Although histological changes of the parotid gland in this stage were so slight, significant alteration of acinar cells could be detected by electron microscopy. The nuclei were mostly oval with occasional indentations and condensed chromatin. Secretory granules of various electron density and the endoplasmic reticulum with dilated cisternae were seen as deviation from normal parotid acinar cells (Fig. 2).

**96 hours after ligation:** The pancreas showed marked dilatation of the central lumen of acini together with cellular dissociation and acinar atrophy. Degranulation of acinar cell was most conspicuous in this stage. Interstitial edema was enhanced in inter- and intra-lobular regions and moderate degree of inflammatory cell infiltration was observed here concurrently with slight proliferation of fibroblasts. The ultrastructural observation revealed marked decrease of secretory granules, appearance of lipid droplets with moderate density, about 1 μ in diameter, in the basal or perinuclear portion of acinar cells. The granular endoplasmic reticulum diminished in amount with dilatation of its cavities. A small number of mitochondria exhibiting a dense matrix and indistinct cristae were irregularly distributed. The inconspicuous Golgi complex showed similar profiles as in control cases (Fig. 3).

An obvious deviation from usual histological appearance of the parotid acinar cells was found in this stage. Central lumens of acini were distended and disarrangement of acinar cells accompanied by atrophic changes of these cells was observed (Fig. 4). The commonest ultrastructural alterations of these cells were condensation of nuclear chromatin, marked decrease of secretory granules, appearance of small vacuoles derived from dilated granular endoplasmic reticulum, appearance of lipid droplets with moderate electron density (Figs. 5 and 6) and dilatation of secretory canaliculi.

**Group of obstructive jaundice**

Changes of the pancreas following the ligation of the common bile duct at the liver hilar portion were much milder than those found in the pancreas following the ligation at the orifice to the duodenum. Only edema and appearance of lipid droplets in basal portion of the acinar cells were noted. Secretory granules remained relatively abundant. The ultrastructural changes seen in acute pancreatitis such as dilatation of the granular endoplasmic reticulum were not noted (Fig. 7).

In the parotid gland, lipid droplets appeared within the acinar cells but no other changes were observed (Fig. 8).

**Group of perforative peritonitis**

No changes of secretory granules or other cellular organelles were noted in the pancreas and parotid gland 24 and 96 hours after operation (Figs. 9 and 10).
Fig. 5. Higher magnification of the parotid gland in Fig 4. The acinar cell contains numerous vesicles of the granular endoplasmic reticulum (Er) and lipid droplets (Li). Original magnification ×6,000.

Fig. 6. Higher magnification of the parotid gland in Fig 4. The acinar cell contains secretory granules (Sg), vesicles of the granular endoplasmic reticulum (Er) and electron dense mitochondria (M). Original magnification ×8,000.

Group of intestinal obstruction

As in the experiments of perforative peritonitis, no remarkable changes were noted in the histology or fine structure of the pancreas and the parotid gland (Figs. 11 and 12).
Fig. 7. Pancreas 96 hours after ligation of the common bile duct at the liver hilar portion. Acinar cells show no significant alterations in organelles except appearance of lipid droplets in the basal portion of the cytoplasm. Original magnification ×5,000.

Fig. 8. Parotid gland 96 hours after ligation of the common bile duct at the liver hilar portion. Acinar cells contain many secretory granules (Sg) and several lipid droplets (Li). Original magnification ×2,500.

**DISCUSSION**

The pancreas and the parotid gland are analogous organs, which secrete amylase and bicarbonate and possess similar histological structure except for the presence of islets of Langerhans in the pancreas (Best and Taylor 1966; Texter
et al. 1968). In a series of our studies concerning relationship between pancreas and parotid gland, the authors reported previously that, when acute pancreatitis was produced experimentally, histological changes took place in the parotid gland, such as atrophy of acinar cells, loss of zymogenic granules and vacuolation of the acinar cell cytoplasm, and that these changes progressed proportionally to the severity of pancreatic lesions (Kakizaki et al. 1971, 1972a, b, c, 1974). Although
these pathological changes have only been demonstrated by light microscopy, the fine structural changes of parotid gland are also of importance for the better understanding of inter-relationship between these two organs.

In the present study the ultrastructural changes of the parotid acinar cells observed in the rats with acute pancreatitis were depletion of zymogenic granules,
dilatation of endoplasmic reticulum, condensation of nuclear chromatin and appearance of lipid droplets. Scott and Pease (1967) noted in their report on cyclical morphological changes of the parotid gland during glandular activity that the endoplasmic reticulum increased in amount following the depletion of secretory granules caused by fasting-refeeding, pilocarpin administration or electrical stimulation. In our study, depletion of secretory granules and dilatation of endoplasmic reticulum in the parotid acinar cells were noted simultaneously, and therefore these may represent degenerative changes, which simulate the so-called "exhausted cell" of glandular cell in general.

Appearance of lipid droplets within acinar cell of the parotid gland equally occurred after ligation of the common bile duct at the orifice to duodenum as well as at the liver hilar portion. Since in the latter experiment no pathological changes except appearance of lipid droplets were observed in either pancreas or parotid gland, the appearance of lipid droplets would not have been caused by metabolic change in the pancreas but rather be due to disturbance of fat metabolism in the liver.

On the other hand, the ultrastructural changes of the parotid gland seen only in the rats with experimentally produced pancreatitis are considered to be induced in some way by acute pancreatitis, for in the cases of experimental perforative peritonitis and ileus such histological and ultrastructural changes were hardly noted in the parotid gland as well as in the pancreas. The results of our present study provide additional evidence for a close relationship between the parotid gland and the pancreas.

References


