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Fine reconstruction of the pancreatic ductular system at the onset of pancreatitis

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Summary. The three-dimensional structure of the pancreatic ductular system (from the intercalated duct to the intercellular secretory canaliculus) is controversial and unclear. The aim of this study is to reveal the three-dimensional structure of the pancreatic ductular sysytem at the onset of pancreatitis. One day following rat pancreatic duct ligation, dilated lumina from the pancreatic ductular system were reconstructed by light microscopic and scanning electron microscopic examination of pancreatic tissue serial sections. The existence of the intra-acinar duct, which is formed only by centroacinar cells and interconnects the adjacent central lumina in an acinus, was demonstrated. The intercellular secretory canaliculi, which are the terminal parts of the pancreatic ductular system, anastomose and end blindly in the intercellular space located between adjacent lateral surfaces of the acinar cells. The intercalated ducts, the intra-acinar ducts, the central lumina, and the intercellular secretory canaliculi are arranged together in a complex connecting and branching system. However, there were no anastomoses found among the central lumina or acini.

Key words: Three-dimensional structure, Scanning electron microscopy, Light microscopy, Reconstruction, Pancreatic ductule

Introduction

Though the pathogenesis of chronic pancreatitis is still unknown, it is certain that an increase of pancreatic duct pressure is an important factor contributing to its onset or advancement. In patients with chronic pancreatitis, light microscopic (LM) examination of the pancreatic tissue demonstrates characteristic ductal changes, which include ductular proliferation and irregularly-dilated pancreatic ducts (bead-like deformity), acini deletion, and fibrosis. Therefore, in order to elucidate the pathogenesis of chronic pancreatitis, successive morphological examinations of pancreatic tissue from onset to advanced stages is very important. In paticular, we believe that the successive ductal changes present in chronic pancreatitis are worth examining. However, on the basis of LM reconstruction of pancreatic tissue, even normal morphological features of the pancreatic ductular system (the area from the intercalated duct to the intercellular secretory canaliculus) is controversial (Bockman, 1976, 1978; Takahashi, 1984; Akao et al., 1986) and unclear, because the central lumen or the intercellular secretory canaliculus are so small 0.6-1.0 μm in diameter (Takahashi, 1984) that it is difficult to recognize their three-dimensional structure by the conventional LM examination. One day after pancreatic duct ligation, we observed serial sections of rat pancreatic tissue, 1 μm thick, using LM and 0.4 μm thick using scanning electron microscopy (SEM). As a result, we were able to reconstruct the dilated lumen of the pancreatic ductular system. It is suggested that these reconstructed lumina demonstrated the three-dimensional structure of pancreatic ductular system at the onset of acute or chronic pancreatitis, while preserving an almost normal morphology.

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Materials and methods

In the present study, a canaliculus formed only by the centroacinar cells is referred to as an intra-acinar duct. The central lumen is defined as a canaliculus formed by the apical surfaces of the acinar cells. The intercellular secretory canaliculus is defined as a canaliculus located between two adjacent lateral surfaces of acinar cells (Ashizawa et al., 1997) (see Fig. 5).

Animal preparation

The use and care of all animals in this study were performed following Law Concerning the Protection and Control of Animals of Shimane Medical University.

Four twelve-week-old male Wistar rats were used: two of them for SEM examinations and the remaining two of them for LM examinations. They were fed with commercially available chow (Charles River Formula-1; Oriental Yeast Company, Tokyo, Japan) and given water ad libitum before and after surgery. The rats were laparotomized along the midline under anesthesia induced by an intraperitoneal injection of sodium pentobarbital (4 mg/100 g body weight). The common hepatic duct was ligated proximal to its entry into the pancreas, and the common bile-pancreatic duct was also ligated near its junction with the duodenum, after which the abdomen was closed.

One day after the operation, the rats were killed by exsanguination under deep anesthesia induced by diethyl ether inhalation. Pancreatic tissue specimens were taken from the area close to the spleen in the splenic lobe, since this portion was the most distant from the ligation point and, therefore, received the least amount of injury from the surgical treatment. One twelve-week-old male Wistar rat was used for the normal control in LM examination.

Preparation of pancreatic tissue for LM reconstruction studies

The pancreatic tissue specimens were immersed in 2.5% glutaraldehyde in a 0.1M phosphate buffer (pH 7.3) at room temperature for 12 hours. They were then washed in a 0.1M phosphate buffer (pH 7.2), and post-fixed with 1% osmium tetroxide in a 0.1M phosphate buffer (pH 7.0) at 4 °C for 2 hours. After dehydration in a graded series of ethanol, they were embedded in epoxy resin (EPON 812 RESIN, TAAB Company).

Reconstruction of pancreatic ductular system serial sections by LM examination

After embedding in epoxy resin, semithin sections (1 µm thick) cut from the specimens were stained with toluidine blue and photographed under LM at a final magnification of x544. In the 26 pancreatic ductular systems examined, the diameters of the long axes of the ductular lumina (from the intercalated ducts to the intercellular secretory canaliculi) in serial sections (50-97) were traced as transverse straight lines on a two-dimensional graph, and an accumulation of these transverse lines, which represented the ductular lumen two-dimensionally, were drawn as an area filled with black on this graph. The acinar cells, the centroacinar cells, and the intercalated duct cells, which surround the ductular lumen, were also reproduced on this graph.

Preparation of pancreatic tissue for SEM reconstruction studies

The specimens of pancreatic tissue were fixed by immersing in the 3.7% formaldehyde solution in a 0.1M phosphate buffer (pH 7.4) at 4 °C for 12 hours. Specimens were washed in a 0.1M phosphate buffer (pH 7.3) and dehydrated by a graded series of ethanol. Ethanol was then replaced with stylen monomer (Oken Syoji, Tokyo). Finally, specimens were immersed in a stylen monomer with 0.5% benzoil peroxide (Oken Syoji, Tokyo) (w/v) as a catalyst. Polymerizing reaction was carried out in a gelatin capsule at 65 °C for 36 hours. Serial semithin sections of pancreatic tissue (0.4 µm thickness) were attached on the glass coverslips, and stylen resin was removed by a chloroform treatment (65 °C, 12 hours). Semithin sections were dried by the t-butyl alcohol freeze-drying method (Inoue and Osatake, 1988), and then spatter-coated with gold for SEM observation.

Reconstruction of pancreatic ductular system serial sections by SEM examination

In each rat, over 500 serial semithin sections (0.4 µm thick) cut from the specimens were examined three-dimensionally at an acceleration voltage of 20 kV using a SEM (S-800, Hitachi Company, Tokyo, Japan). The pancreatic ductular system was recognized as the continuous lumen provided with microvilli, which are finger-like processes (about 0.1 µm in diameter). The outline of the pancreatic ductular lumen in each section was traced onto transparent paper. In 10 pancreatic ductular systems, the continuity, the end, or the joint of their lumina in serial sections were schematically represented by a two-dimensional line. The joint of two adjacent ductular lumina was distinguishable from one of their blind alleys, because the diameters of the peripheral ductular lumina were more than 0.6 µm (Takahashi, 1984) in each section (0.4 µm thick).

Results

LM examination of the pancreatic tissue semithin sections

One day after pancreatic duct ligation, the normal lobular architecture was found to be preserved in spite of interstitial edema, a slight infiltration of inflammatory cells, dilatation of the pancreatic ductular system, and an increased number of zymogen granules in acini. The dilated central lumina were 2.5-7.3 µm in diameter, while in normal control, most central lumina were 0.7-1.9 µm. Some central lumina were formed only by acinar cells, and others were formed by acinar cells and centroacinar cells. Furthermore, intra-acinar ducts, which were formed only by the centroacinar cells, were observed (Fig. 1).

LM reconstruction of serial sections of the pancreatic ductular system

Some non-branched intercalated ducts, which interconnected two acini, were found. In the acini, the intra-acinar ducts were joined to either the intercalated

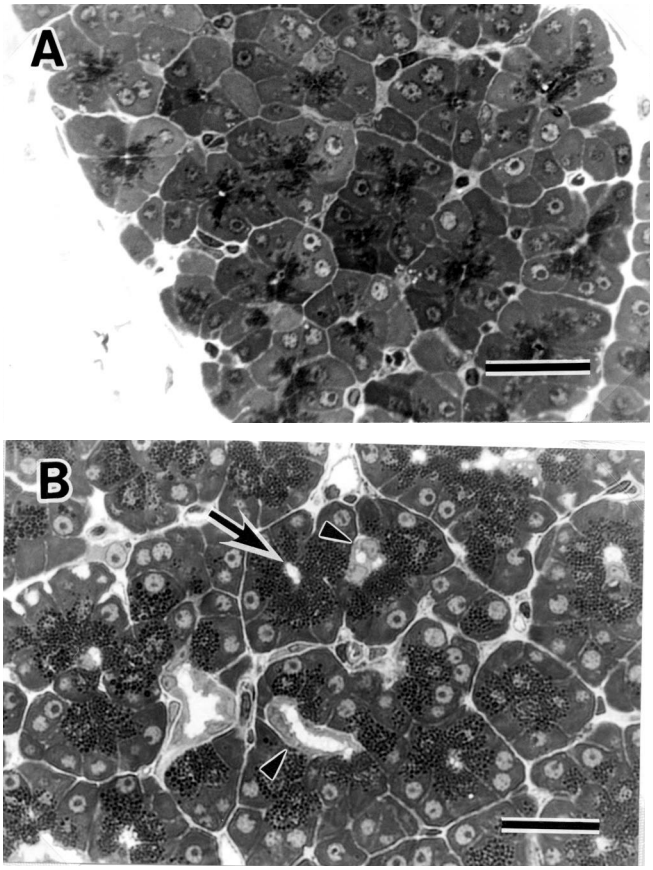

Fig. 1. Light microscopic view of a 1.0 µm-thick section of pancreatic tissue in the normal control rat **(A)** and the rat one day after pancreatic duct ligation **(B)**. Note the intra-acinar ducts (arrowheads) formed only by centroacinar cells in B and the dilated central lumen (arrow) formed only by acinar cells with many zymogen granules. Toluidine blue stain, x 400, Bar: 25 µm.

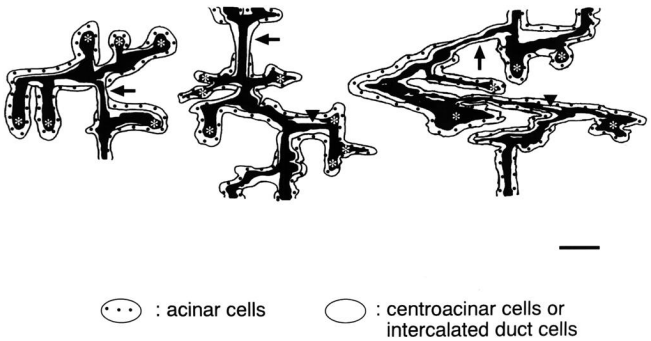

Fig. 2. Two-dimensional schemata of the reconstructed pancreatic ductular system by light microscopic examination of 1.0 µm-thick pancreatic tissue serial sections one day after pancreatic duct ligation. The ductular lumina are shown as the area filled with black. Asterisks indicate blind alleys of the ductular lumina. Every blind alley is formed by the apical surfaces of acinar cells. Arrows indicate non-branched intercalated ducts, which interconnect two acini. Note the intra-acinar ducts interconnecting adjacent central lumina (arrowheads). Bar: 20 µm.

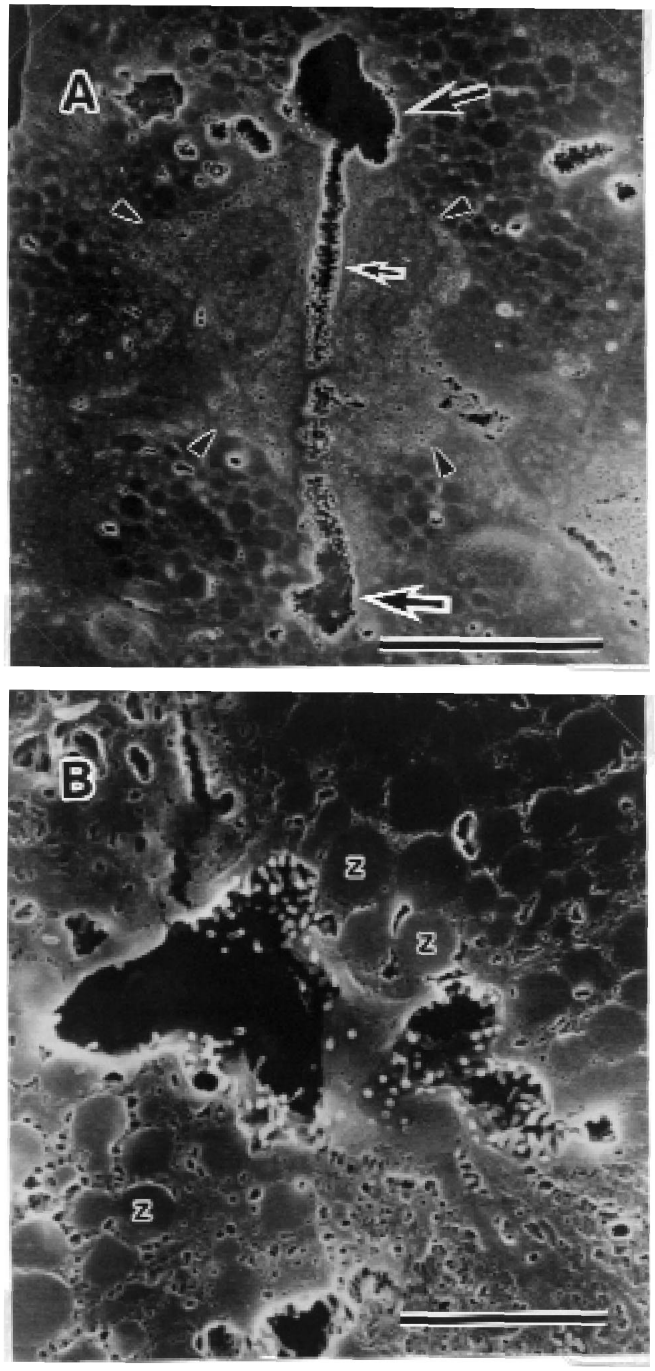

Fig. 3. SEM view of a 0.4 µm-thick section of pancreatic tissue one day after pancreatic duct ligation. **A.** An intra-acinar duct (small arrow), which is formed only by centroacinar cells (arrowheads), interconnects two adjacent central lumina (large arrows), which are formed by the apical surfaces of acinar cells. By examination of the serial sections, it is demonstrated that this intra-acinar duct (small arrow) has no branch. x 3,000, Bar:10 µm. **B.** Higher magnification of the central lumen, which has finger-like microvilli on the inner surface. Z indicates a zymogen granule located in the vicinity of the central lumen. x 10,000, Bar: 3 µm.

ducts or interconnected with adjacent central lumina (Fig. 2). Every blind alley in the ductular lumen was surrounded by acinar cells. That is to say, every terminal part of the exocrine pancreas was an acinus, and blind alleys of the ductular lumina were the central lumina. Among the acini, the intra-acinar ducts, or the central lumina, no anastomoses were found.

SEM examination of semithin sections of pancreatic tissue

The centroacinar cells and intercalated duct cells were distinguishable from the acinar cells, since they had no zymogen granules even in the portion close to the lumen (Fig. 3A). The pancreatic ductular system was recognized by observing the luminal surface containing finger-like microvilli (Fig. 3B). Though diameter of the central lumen was more than 2.0 µm, the intercellular secretory canaliculus was less than 1.0 µm.

SEM reconstruction of serial sections of the pancreatic ductular system

The intercellular secretory canaliculi were arranged in a complex branching system. In some acini, an intercellular secretory canaliculus extending from its blind alley toward the intercalated duct branched into two or three canaliculi, which were located at intervals of less than 2.0 µm, before rejoining at a canaliculus (Fig. 4). In a few acini, a non-branched intra-acinar duct was seen interconnected with adjacent central lumina (Fig. 3A).

Discussion

On the basis of LM reconstruction of normal pancreatic tissue, Bockman (1976, 1978) and Akao et al. (1986) reported observing ductules and acini arranged in a complex, branching system of tubules, which anastomosed and ended blindly, whereas Takahashi (1984) noted that there were no anastomoses seen among the intercalated ducts and acini. However, it is difficult to discern the three-dimensional structure of the intercellular secretory canaliculus, the central lumen, and the peripheral intercalated duct by the conventional LM examination of 3-10 µm-thick pancreatic tissue specimen, since their lumina are so small. In the present LM study, the central lumen or intercellular secretory canaliculus were about 1.0 µm (0.7-1.9 µm) in diameter. In our previous transmission electron microscopic (TEM) study, the lumen of intercalated duct was about 2.0 µm (1.00-4.05 µm) (Endo and Ashizawa, 1997). Though our SEM observation of pancreatic duct corrosion casts demonstrated the existence of anastomoses between the intercalated ducts (Ashizawa et al., 1991, 1997), we could not demonstrate the peripheral intercalated ducts, central lumina, or intercellular secretory canaliculi, due to leakage of the casting medium out of the pancreatic ductular system (Ashizawa et al., 1991). Indeed, with conventionally prepared pancreatic tissue, SEM observation of the pancreatic ductular system lumina was hampered by the protein contents in the pancreatic juice (Ashizawa et al., 1991), and TEM reconstruction of serial sections only 0.1 µm thick is very difficult. However, we have noticed by SEM observation of pancreatic tissue that after pancreatic duct ligation, the dilated lumina of the pancreatic ductular system have very little protein

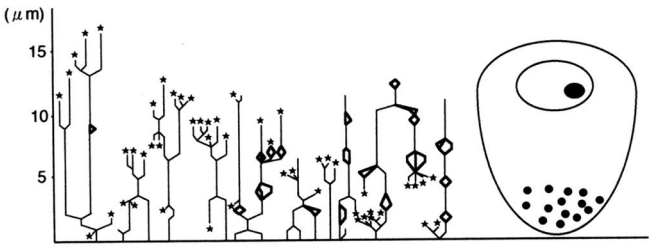

Fig. 4. Two-dimensional schemata of the reconstructed pancreatic ductular system by SEM examination of 0.4 µm thick pancreatic tissue serial sections one day after pancreatic duct ligation. Lines denote the continuity or joint of the ductular lumina. Thick lines indicate anastomoses. The acinar cell schema express the size. Asterisks indicate blind alleys of the ductular lumina. Some intercellular secretory canaliculi can be seen extending from blind alleys toward the intercalated ducts, and then branching into two or three intercellular secretory canaliculi, which are located at intervals of less than 2.0 µm, before rejoining an intercellular secretory canaliculus.

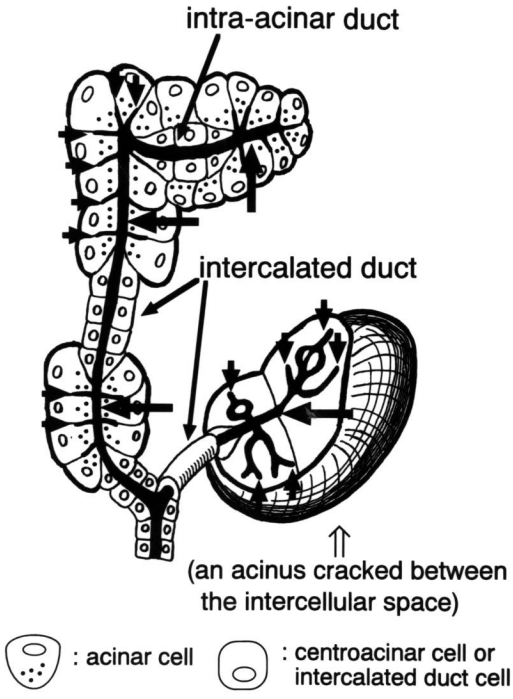

Fig. 5. Schema of the peripheral exocrine pancreas morphology. The ductular lumina are shown as the area filled with black. Large arrows indicate the central lumina. Small arrows indicate intercellular secretory canaliculi. Thick lines indicate the basement membrane of acini.

content. In rats and mice, pancreatic duct ligation for three days induces atrophy of the pancreatic tissue subsequent to mildly acute edematous pancreatitis, which includes ductular proliferation caused by deletion of the acinar cells due to apoptosis and proliferation of the ductular cells (Boquist and Edstoem, 1970; Pound and Walker, 1981; Walker, 1987; Walker et al., 1992; Watanabe et al., 1995 ; Doi et al., 1997; Wada et al., 1997). In the present study, we demonstrated the three-dimensional structure of the rat pancreatic ductular system by LM and SEM reconstructions of serial sections of pancreatic tissue one day after pancreatic duct ligation. LM observation of pancreatic tissue demonstrated that an almost normal lobular architecture was preserved, in spite of the dilated pancreatic ductular system and the interstitial edema, one day after ligation. It is suggested that these reconstructed lumina demonstrated the three-dimensional structure of the pancreatic ductular system at the onset of acute or chronic pancreatitis, while preserving an almost normal morphology. However, the intercellular secretory canaliculi could not be demonstrated by LM examination, since their lumina were not so dilated (less than 1.0 µm).

The present study revealed the following findings: 1) there were no anastomoses found among the acini; 2) some non-branched intercalated ducts interconnected two acini; 3) in the acinus, the non-branched intra-acinar duct interconnected with adjacent central lumina; and 4) in some acini, an intercellular secretory canaliculus extending from its blind alley toward the intercalated duct branched into two or three canaliculi, which were located at intervals of less than 2.0 µm, before rejoining a canaliculus. Since the acinar cells were more than 10 µm in size (Fig. 4) , it is further suggested that the anastomosing canaliculi observed were the intercellular secretory canaliculi, and not the central lumina or intra-acinar ducts. Therefore, the intercellular secretory canaliculi were found arranged in a complex branching and anastomosing system in the intercellular space between adjacent lateral surfaces of the acinar cells (Fig. 5).

It is certain that an increase in pancreatic duct pressure is an important factor contributing to the onset or advancement of chronic pancreatitis. During the increase in pressure, the most dangerous portion anatomically is the interval space between the end of the intercellular secretory canaliculus and the acinar basement membrane. This portion has the narrowest interval between the inner surface of the pancreatic ductular system and the basement membrane (Takahashi, 1984), and pancreatic juice is liable to leak out of the pancreatic ductular system into the interstitial tissue. Apoptosis of the acinar cells causes deletion of the most dangerous portions and a decrease of the zymogen enzyme. On the other hand, the pancreatic ductule may stand up to increased pancreatic duct pressure by hyperplasia of the duct cells. We suppose that the intercalated duct interconnecting the acini and the intra-acinar duct enables a prompt change from a normal lobular architecture to ductular proliferation, which is a structure more resistant to an increase of pancreatic duct pressure.

In summary, the intercalated ducts, the intra-acinar ducts, the central lumina, and the intercellular secretory canaliculi were found arranged in a complex connecting and branching system. The intercellular secretory canaliculi, which are the terminal pancreatic ducts, were seen to anastomose and end blindly in the intercellular space between the acinar cells. However, there were no anastomoses observed among the central lumina or acini. (Fig. 5).

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