An experimental study in etiologic effect of pancreas divisum on chronic pancreatitis and its pathogenesis *

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Subject headings pancreatitis/etiology; pancreatitis/physiopathology; pancreas divisum/physiopathology; pancreas divisum/etiology; chronic diseases

Abstract
AIM To investigate the etiologic association of pancreas divisum (PD) with chronic pancreatitis and to clarify its pathogenesis.

METHODS A PD canine model was established in 32 dogs. The dogs were randomly divided into 4 groups (n=8). Group I: The communicating branch between the dorsal and ventral pancreatic ducts was partly ligated. Group IIa: The communicating branch was amputated and completely ligated. Group IIb: The dorsal duct was amputated and ligated at 2mm distance to the minor papilla. Group III: A sham operation without any amputation or ligation was performed. Before and after operation, the activities of serum phospholipase A2 (PLA2) and amylase (Ams) were assayed and the basal pressures of the ducts were measured when secretin was injected. Pancreatic ductography and the pathologic examination were made.

RESULTS The activities of serum PLA2 and ams in Group I, IIa, and IIb were significantly increased 5 - 80 days after operation. At sacrifice, the basal pressures of the ventral duct were significantly wiaher 30min-60min after provocation in Group I, IIa and IIb. The pressures of the dorsal duct were significantly increased in Group IIb but no difference in Group I and IIa. Under light microscopy the fibrosis of interlobus and periducts, the destruction of acini and infiltratiob of inflammatory cell in dorsal and ventral pancreas were found in Group IIb. But in Group I and IIa, this findings were present only in ventral pancreas. The electron microscopy showed that in ventral pancreas of Group I and IIa and the dorsal and ventral pancreas of Group IIb, the rough endoplasmic reticulum of the acinar cells showed granules-scaling, fusion and dilatation. The zymogen granules decreased and the mitochondria was swollen.

CONCLUSION PD is one of etiologic factors in chronic pancreatitis. The pathogenesis is the functional obstruction of the minor papilla at the peak stage of secretion.

INTRODUCTION
The patients with pancreas divisum (PD) were considered to have a higher risk for chronic recurrent pancreatitis. But the ant the etiology and pathogenesis are still unclear. We established a canine model of PD in 32 dogs for investigating the etiologic association and clarifying the pathogenesis.

MATERIALS AND METHODS
Animal model
Thirty-two healthy adult dogs of both sexes weighing about 10kg were used. Prior to the experiments, they were fasted for 24 hours. Under the sodium pentobarbital anesthesia, an abdominal midline incision was made and the head of pancreas was exposed. The partial ligation or amputation of the pancreatic duct was randomly made in 4 groups. Group I: the communicating branch between the dorsal and ventral pancreatic ducts was partly ligated at the middle segment (n = 8). Group IIa: the communicating branch was amputated and the remaining stump was ligated (n = 8) Group IIIb: the dorsal pancreatic duct was amputated and ligated at a 2-mm distance from the minor papilla (n = 8).

Pancreatic enzymes assay
The activities of serum phospholipase A2 (PLA2) and amylase (Ams) were assayed before ligation
and 5, 10, 15, 30, 50, 80, 120, 150 and 180 days after operation by the method of radioimmunoassay and blue starch respectively.

**Manometry of pancreatic duct**
The pressure of the dorsal and ventral pancreatic duct was measured before ligation and at sacrifice at 0, 15, 30, 60 and 90 min after intravenous injection of secretin (Kabi Vitrum, Stockholm, Sweden) at a dose of 11 U/kg. A polyvinyl catheter with a 0.8mm inner diameter and 1.0mm outer diameter was used. The probe contained an end orifice measuring 0.8mm in diameter and was filled with sterile saline. The perfusion took place at a constant rate of 0.25ml/min. The pressure was recorded on a thermal pen recorder (Nippon Sanei 360, Tokyo, Japan).

**Pancreatic ductography**
Pancreatography was performed by retrograde perfusion via the major and minor papilla cannulation with 180mgI/ml Omnipaque (Nycomed Co, Norway) before operation and at sacrifice for investigating the pancreatic ductal changes.

**Tissues preparation for light and electron microscopy**
The specimens for light microscopy were fixed with 10% formalin solution and embedded with paraffin wax. The sections were HE stained. Blocks of tissues (1.0mm³) cut from pancreas and duodenal papilla for electron microscopy were fixed with 2.5% glutaraldehyde, postfixed with 1.0% OsO₄ dehydrated with ethanol, and embedded with Epon 812. The sections stained with uranyl acetate and lead citrate were examined under the Hitachi H-600 electron microscopy.

**Statistical analysis**
Results were expressed as mean±SD. Comparisons among groups were made using Scheffe’s multiple test or Spearman’s rank correlation analysis.

**RESULTS**

**Biochemical assay**
In Group IIb, the activities of the serum PLA2 and Ams were significantly increased after operation, especially during the postoperative 15-80 days. The activities in Groups I and Group IIa were slightly increased during the postoperative 5-15 days, but there was no statistically difference as compared with preoperation. There were no changes in the activities in Group III before and after operation.

**Manometries of pancreatic duct**
Before operation, the basal pressures of the dorsal and ventral duct were 0.78 kPa ± 0.21 kPa and 0.69 kPa ± 0.24 kPa, respectively. When the secretin was administrated, the were slight increased at 30 and 60 min, but with no statistical difference (all P>0.05). During 60min-90min, the pressures reawiled at the preoperative levels.

**At sacrifice**
In ventral duct, the basal pressures in Groups I, IIa and III were 0.80 kPa ± 0.3 kPa, 0.79 kPa ± 0.28 kPa, 0.64 kPa ± 0.20 kPa and 0.83 kPa ± 0.24 kPa, respectively. There was no significant difference as against preoperation. After secretin injection, the pressure at 30 min was significantly higher than that before operation in Groups I, IIa and IIb. At 60 min, the pressures in Groups I and IIa returned to the preoperational level at 90 min. In dorsal duct, the pressures were significantly increased in Group IIb (P>0.001). In Groups I, IIa and III, there were no significant differences before and after secretin injection.

**Pathological changes**
Light microscopy. In ventral pancreas, interlobus or and periductal fibrosis, the destruction of acini and infiltration of inflammatory cell were found in Group IIb, slight periductal fibrosis in Groups I and IIa, and no abnormal histological changes in dorsal pancreas in Groups I and IIa, but chronic pancreatitis was present in Group IIb. The duodenal papilla was histologically normal.

Electron microscopy. The rough endoplasmic reticulum of the dorsal and ventral pancreatic acinar cells in Groups I, IIa and IIb showed granules-scaling, fusion and vacuolar dilatation. The Zymogen granules decreased and their electron density reduced. The mitochondria were swollen and the space around the nucleus was increased.

**DISCUSSION**
PD had been recognized by the 17th century anf it was found in approximately 7% of western autopsies. A higher incidence of chronic pancreatitis was discovered among the patients with PD. But their etiologic association has not been established, because so far there has been no animal models concerning PD. We established the animal model in 32 dogs through partial ligation or amputation of the communicating branch between the dorsal and ventral pancreatic duct. Not only the anatomic shapes but also the pathophysiology bases were similar to that in human. PLA2 presents in the pancreas as an inactive zymogen, and inappropriate
Intrapancreatic activation of PLA2 is thought to play an important role in the development of pancreatitis. In this study, the PLA2 activities in Groups IIa and IIb, especially in Group IIb, were significantly increased, indicating the possibilities of pancreatitis. It was reported that PLA2 activities could be used to evaluate the severity of acute pancreatitis\textsuperscript{[3]}. In Group IIb, due to all the pancreatic secretions occurring through the major papilla, not as the other groups, it was not surprising that more severe pancreatitis was induced.

The previous studies concerning the drainage of the pancreatic secretions were to measure the diameter of the duct by means of CT or ultrasound during secretin provocation\textsuperscript{[4,5]}. It was not accurate because dilatation occurred in the pancreatic ducts when the periductal fibrosis was obvious. In the present study, the pressures of the ventral duct in Group IIb at 30 and 60min were significantly higher than that before provocation. It implied the existence of physiologic obstruction. Chronic ventral and dorsal pancreatitis was found in Group IIb. But the similar changes were not seen in Groups I and III, and slight in Group IIa, and the duodenal papilla was histologically normal, confirming the association of pancreatitis with relative obstruction of secretion drainage. In Groups I and IIa, the communicating branch between the ventral and dorsal ducts were partly ligated or amputated. The juice secreted from the dorsal and ventral pancreas drained from the minor and major papilla, respectively. So, it is not likely to block the drainage. In Group IIb, all of the secretions drained via the major papilla. To block the drainage at the peak stage of the secretion was not avoided. Therefore, we have come to the conclusion. That there was clear etiologic association between PD and chronic pancreatitis.

**REFERENCES**

1. Warshaw AL, Simeone JF, Schapiro RH, Warshaw BF. Evaluation and treatment of the dominant dorsal duct syndrome (pancreas divisum redefined). *Am J Surg*, 1990;159(1):59-66
2. Lu WF, Xu P, Zhu CR, Wu BH. The ERCP diagnosis and its significance of pancreas divisum. *Chin J Radiol*, 1996;30(3):175-178
3. Kitagawa M, Hayakawa T, Kondo T, Shibata T, Sakai Y, Seabajima H et al. The diagnosis value of serum pancreatic phospholipase A2 in pancreatic diseases. *Gastroenterol Japonica*, 1991;26(1):62-64
4. Lindstrom E, Ihse I. Dynamic CT scanning of pancreatic duct after secretin provocation in pancreas divisum. *Dig Dis Sci*, 1990;35(10):1371-1378
5. Warshaw AL, Simeone J, Schapiro RH, Hedberg SE, Muller FE, Ferrucci JT. Objective evaluation of ampullary stenosis with ultrasonography and pancreatic stimulation. *Am J Surg*, 1985;149(1):65-72