A new animal model for local pancreatitis, ventral pancreatic duct ligation in the adult house musk shrew, *Suncus murinus*

**ABSTRACT**

Various animal models for pancreatitis, using large or small animal models, have been established to study pathophysiology, structure, diagnosis, and treatment of pancreatic disorders. Obstruction of the pancreatic duct is considered to play an important role in the progression of acute and chronic pancreatitis in humans. For this reason, many studies using pancreatic duct ligation animals have been reported. The right lobe of pancreas in *Suncus murinus* unlike other animals such as rat and mouse is supplied by independent pancreatic duct, vessels and innervation, and similar to the ventral pancreas in human embryo. The characteristic can be utilized as a model to study the mechanism of local pancreatitis and pancreatic fibrosis. In this study, the pancreatic duct of right lobe was ligated selectively in *Suncus*. Inflammatory cell infiltration, irregular widening and dilations in the pancreatic duct and/or around the surrounding tissue appeared in the early stage and the fibrosis signs were detected in two-three weeks after ligation operation. The related factors of pancreatic fibrosis, α-SMA and collagen type I were assayed by immunohistochemistry. In conclusion, the present experiment was designed to ligate selectively the ventral pancreatic duct to establish a ventral pancreatitis and pancreatic fibrosis model by using *Suncus* special anatomical structure of pancreas. The procedure of ligation was simple, not to cause any harm to the surrounding tissues, it is an available experimental model that can be used to study this mechanism of pancreatitis and pancreatic fibrosis.

**Key words:** Pancreatic duct ligation; ventral pancreas; pancreatic fibrosis; pancreatitis model; immunohistochemistry; *Suncus murinus*.

**INTRODUCTION**

Various animal models for pancreatitis, using large or small animal models, have been established to study pathophysiology, structure, diagnosis, and treatment of pancreatic disorders. All these models were induced by surgical procedures, such as pancreatic duct ligation (PDL) and closed duodenal loop technique (Araki et al., 2003), as well as by injecting various chemical substances, such as dibutyldichlorotin (DBTC) (Sparmann et al., 1997), trinitrobenzene sulfonic acid (TNBS) (Xu et al., 2006), L-arginine (Toma et al., 2000), cerulein (Shimizu et al., 1993) or micro-spheres, intravenously, intraperitoneally, and intraductally. Obstruction of the pancreatic duct is considered to play an important role in the progression of acute and chronic pancreatitis in humans. For this reason, many studies using PDL animals have been reported (Shay et al., 1953; Churg et al., 1971; Kimura et al., 1996; Aghdassi et al., 2011; Möessler et al., 2012).

PDL method can be easily performed in big animals, e.g.
rabbit (Arvanitakis and Folscroft, 1978), chick (Rideau et al., 1985; Martland, 1986), pig (Kurahashi et al., 2004; Prykhodko et al., 2014), cat (Zhang et al., 2013), dog (He et al., 1998; Nagaya et al., 2004), however, it is very difficult to try this model in small animals (Miyasaka et al., 1992; Yasuda et al., 1999), especially, ligated selectively the pancreatic duct. Usually in small animal model, ligation or obstruction of the pancreatic duct was performed combining ligation of the bile duct (Kimura et al., 1996; Yoshinaga et al., 2000; Miyauchi et al., 2007).

In our previous studies (Yi et al., 2003b), we examined anatomical details of the pancreas in the house musk shrew, Suncus murinus, one of the most common insectivores. The adult Suncus pancreas is clearly separated into right and left lobes that are not fused. The right lobe is located in the dorsum of the duodenum and to the right of the common bile duct independently, and supplied by branches of the superior mesenteric artery. The right lobe in Suncus corresponds to the duodenal lobe in rats or mice (Kishi et al., 2003), which is called right pancreas in this study. The left lobe occupies 9/10 of the entire pancreas and is located to the left of the common bile duct. It is supplied mainly by branches of the splenic and common hepatic arteries (Figure 1) (Yi et al., 2003a). The left lobe correspond to the gastric and splenic lobes in rats or mice (Kishi et al., 2003), which is called left pancreas in this study. Furthermore, according to our previous studies on the distribution of endocrine cells in Suncus pancreas (Yi et al., 2004) and the blood supply and innervation of the human pancreas (Yi et al., 2003a), embryologically, the right and left pancreas in Suncus correspond to the pancreatic parts derived from the ventral and dorsal pancreatic buds, respectively. In addition, the proximal part of the right pancreatic duct is separated from the vessels of the right pancreas, and directly joins the common bile duct. The Suncus pancreas is a suitable experimental model for studying the mechanism of pancreatic disease and development of the human pancreas (Yi et al., 2003b), especially, as selective pancreatic duct obstruction model.

In this study, we employed the Suncus, performed easily a selective ligation of the right pancreatic duct, and established a new animal model of pancreatitis, which a local chronic pancreatitis and fibrosis model, and one part of the pancreatic tissue is damaged and another part of the pancreatic tissue is normal in the same animal.

MATERIALS AND METHODS

Animals

Adult Suncus were obtained from a closed colony bred in our laboratories. The mother colony, JIc: CR, is maintained in the Central Institute for Experimental Animals, Kawasaki, Japan (Yi et al., 2006, 2007). The animals were housed and handled in accordance with the Guide for the Care and Use of Laboratory Animals and the Guide for the Care and Use of Experimental Animals of the Canadian Council on Animal Care. Briefly, all shrews were kept individually after weaning (20 days after birth) in plastic cages equipped with a wooden nestbox containing paper strips, and were kept in a conventionally conditioned animal room: 23 to 27°C, no humidity control and 14:10 light:dark. Commercial trout pellets containing 45.0% protein, 3.5% fat, 3.0% fiber, 13.0% ash and 26.2% complex carbohydrate (Nippon Haigou Shiryou, Tokyo, Japan) and water were supplied ad libitum.

PDL model

Adult Suncus (n=66, 36 females and 30 males) weighing between 33.46 and 89.42 g (average 58.33 g) were divided into three groups randomly: PDL group (n=30), sham-operated group (n=18), and no-operation normal (NON) group (n=18). The PDL group was first anesthetized with diethyl ether, and then given an intraperitoneal injection of Somnopentyl (Pentobarbital sodium, 0.6 ml/kg). After the animals were completely narcotized, the abdominal cavity was opened in a midline abdominal incision in 2 cm. Then laparotomy was performed under a dissecting microscope (OLYMPUS, SX16, Japan) to expose the pancreas and its surroundings. The right pancreatic duct was massively-ligated with 7-0 polypropylene suture (ETHICON), while the common bile duct and the vessels of the right pancreas were carefully not ligated or damaged (Figure 1). The left pancreatic duct was left out of the process. Subsequently, the peritoneal lining and the fascia of the rectal abdominal muscle, as a single layer, and the skin was closed. All the procedures were performed under aseptic conditions and it took about 15-20 min (skin to skin). Anesthesia management during the operative procedures and subsequent postoperative care were consistent with the National Institutes of Health guidelines for the care and use of laboratory animals.

After operation, commercial pellets and water were supplied ad libitum until sacrificed. Sham-operated group were subjected to the same procedures but without ligation of the right pancreatic duct. The no-operation normal group that did not undergo any operation was considered as control.

The following protocol was approved by the Animal Care and Use Committee of the Tokyo Medical University.

Preparation of tissue sections

At the following intervals: 2, 5, 7, 10 days and 2, 3 weeks after operation, experimental animals operated were again anesthetized with diethyl ether and then given an intraperitoneal injection of Somnopentyl (Pentobarbital...
sodium, 0.6 ml/kg). After the animals were completely narcotized, the abdominal cavity was opened, and a catheter was inserted retrogradely into the abdominal aorta at the level immediately above the bifurcation of this artery into the common iliac arteries. Perfusion was commenced with 0.01 M phosphate-buffered saline (PBS) (pH 7.4), and thereafter with 4% paraformaldehyde (PFA) buffered with PBS. Thereafter, the right and left pancreas were removed. The samples were post fixed in the same buffer overnight at 4 °C. Normal and sham-operated groups were also performed as stated above.

The samples were dehydrated with ethanol and embedded in paraffin. Paraffin sections (5 µm thick) were placed on slides pretreated with 3-aminopropyltriethoxysilane (Superfrost; Matsunami, Japan) and stored at room temperature (RT) until further processing (immunohistochemistry).

Analysis of pancreatic fibrosis by Immunohistochemistry

Immunohistochemical procedures were performed as previously described (Yi et al., 2010). Briefly, after rinsing the fixed tissue specimens in 0.01 M PBS (pH 7.4), endogenous peroxidase activity was inhibited by 30-min incubation in methanol containing 3% (v/v) hydrogen peroxidase. After rinsing in PBS, the sections were blocked with Protein Block Serum-Free (DAKO Cytomation, x0909, USA) for 1 h at RT, incubated with the primary antibody overnight at 4 °C in a humidified chamber, and then with the secondary antibody for 1 h at RT. Subsequently, the avidin-biotin-complex technique (ABCComplex/HRP; DAKO, k355, Denmark) was performed by incubating the sections with ABC complexes for 30 min at RT, and then treating them with 3,3-diaminobenzidine and 0.005% H2O2, which acted as chromogens. The samples were rinsed in PBS five minutes for three times in every step above. The sections were counterstained with Harris hematoxylin for 50 s, dehydrated in a graded ethanol series and xylene, and mounted under coverslips with Entellan neu (Merck, Dannstad, Germany). The primary antibodies were mouse anti-human α-smooth muscle actin antibody (monoclonal, A2547, Sigma, Missouri, USA), which is a marker of myofibroblast formation, and goat anti-human collagen α1 type I antibody (polyclonal, sc-25974, Santa Cruz), which is one of the markers of fibrosis, diluted at 1:200 and 1:200, respectively. The secondary antibodies were biotin-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology) and biotin-conjugated guinea pig anti-goat IgG (Santa Cruz Biotechnology), respectively.

The control experiments were performed as follows: (1) removal of primary antiserum; (2) substitution of primary antibody with 0.05 M Tris-BSA buffer. The controls were run on sections at the time of treating with the primary antibody.

RESULTS

The right pancreas in sham-operated and in no-operated normal groups, or the left pancreas showed normal pancreatic tissue (Figure 2A).

In the early stage after the PDL operation, it revealed irregular widening and dilations of pancreatic duct, inflammatory cell infiltration around the pancreatic duct in
the ligated-right pancreas (Figure 2B). Moreover, dilatation of small branches of the pancreatic duct and inflammatory cell infiltration became prominent in the ligated right pancreas in 7-10 days after the operation.

Two weeks after the PDL operation, microscopically, atrophic, omission, and vacuolization of acinar cells increased. Extensive fibroblast proliferation became prominent, and fibrosis around the pancreatic duct, duct-like structures appeared conspicuously in the ligated-right pancreas (Figure 2C).

Three weeks after the PDL operation, the ligated-right pancreas showed considerable degeneration and severe and widespread fibrosis, while less exocrine pancreatic tissues were scattered and islets of Langerhans were isolated in the fibrotic tissues (Figure 2D).

Neither necrotic nor hemorrhagic pancreatitis was observed during this experiment.

No expression of α-SMA in right pancreas was detected except on normal vessel walls in the specimens from sham-operated and no-operated normal groups, or one day after the PDL operation (Figure 3A). On the 7th day after the PDL operation, more expression of α-SMA was found in the periductal areas and was increased and extended to interlobular areas on the two (Figure 3B) and three weeks.

Furthermore, positive response cells of collagen Type I was hardly detected in the interlobular areas two days after operation (Figure 3C). And the expression of collagen type I increased around the interlobular areas 10 days after the PBL operation. Fibrosis filled overall the right pancreas two and three weeks (Fig. 3D) after PBL operation.

**DISCUSSION**

In the present study, we performed PDL of the right pancreas in Suncus, an experimental model of a local chronic
pancreatitis and pancreatic fibrosis was established. This model showed a pancreatic duct dilatation and inflammatory cell infiltration, atrophy of acinar cells and vacuolization in acinar cells in early stage after the PDL operation, and the fibrosis signs occurred after 7-10 days. In 2-3 weeks of the operation, the lobules were occupied by duct-like structures (also called the tubular complex) after disappearance of the acinar cells, extensive and severe fibrosis formed. The pathological process of this PDL model was commonly seen in human chronic pancreatitis. PDL method can be performed relatively easier in big animals (Zhang et al., 2013; Prykhodko et al., 2014), however, it is difficult to try this model in small animals. In this experiment, we ligated selectively the right duct of the duodenal lobe only by a 7-0 polypropylene suture on a surgical microscope and left the left duct intact in Suncus. Biochemically, this model can also induce damaged pancreatic tissue in one part and normal pancreatic tissue in another part of the pancreas in the same animal.

Many experimental studies of pharmaceutical treatment for pancreatic insufficiency have been reported. However, it is very hard to evaluate the efficacy of these drugs, especially those given orally, because food consumption in each animal is different (Liao et al., 2010). Our established new models overcome this problem and make it easy to study the effect of pharmaceutical treatment for pancreatic insufficiency.

Fibrosis is a major characteristic and one of the important pathological changes in chronic pancreatitis induced by pancreatic duct obstruction in humans (Perumal et al., 2013). As shown in the present study, the fibrous tissue becomes remarkably rich in the atrophied pancreas after duct ligation. Collagen type I-positive cells, one of the markers of fibrosis, gradually occurred in 1-3 weeks after ligation operation.

In previous reports, the animal models of the main
pancreatic duct ligation reducing chronic pancreatitis have been reported (Catala et al., 1990; Satake and Hiura, 1998). In clinically significant pancreas divisum, the dorsal pancreas nearly always demonstrates radiologic and histologic evidence of pancreatitis, while the ventral pancreas is normal (Brinberg et al., 1988). In the International Workshop held at Middlesex Hospital in 1984, only 6 of 93 patients were judged to have abnormal ventral ductograms with normal dorsal ductograms (2 patients were alcoholics) (Cotton, 1985). Thereafter, more chronic pancreatitis isolated to the ventral pancreas have been reported repeatedly (Brinberg et al., 1988; Saltzberg et al., 1990; Ng et al., 1994; Hara et al., 2013; Gurram et al., 2014). According to our previous studies (Yi et al., 2003a; 2003b; 2004), the right pancreas in Suncus corresponds to the ventral pancreas embryologically and uncinate process of the pancreas in humans. Therefore, this experimental model can be employed to explore the mechanism of the ventral pancreatitis in humans.

Conclusions

The present experiment was designed to ligate selectively the ventral pancreatic duct to establish a ventral pancreatitis and pancreatic fibrosis model by using Suncus special anatomical structure of pancreas. The procedure of ligation was simple, not to cause any harm to surrounding tissue, it can also be as a model to study the mechanism of pancreatitis and pancreatic fibrosis, especially, a local ventral pancreatitis and fibrosis. Further investigation is needed in order to elucidate the effects of islet of Langerhans cells, especially, pancreatic polypeptide cells which are mainly distributed in the right pancreas in Suncus.

CONFLICTING INTERESTS

The authors declare that there are no conflicting interests.

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REFERENCES


