

Role of fibrosis-related genes and pancreatic duct obstruction in rat pancreatitis models: Implications for chronic pancreatitis

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Summary. Human chronic pancreatitis is characterized by irreversible fibrosis, whereas pancreatic fibrosis in animal models is reversible. In this study, we compare the development of pancreatic fibrosis in the dibutyltin dichloride (DBTC) model, WBN/Kob rats and bile duct-ligated (BDL) rats. DBTC (8 mg/kg) was administered to LEW rats, and the pancreas was histopathologically investigated sequentially. Male and female WBN/Kob rats aged 4, 6 and 8 months were also examined. BDL rats were prepared by ligation of the bile duct at the duodenal portion and sacrificed at 3 or 7 days after ligation. Fibrosis in the DBTC model peaked after 1 week and was limited to the areas around the pancreatic ducts after 2 weeks, and was composed of both type I and type III collagen. In contrast, fibrosis in male WBN/Kob rats peaked at age 4 months, expanded into intralobular area, and was composed of type III collagen. It exhibited almost no type I collagen and a marked tendency to regress. Pancreatic fibrosis in BDL rats was somewhat difficult to induce and required increased stimulation. This suggests that fibrosis in human biliary pancreatitis may gradually form based on weak, continuous stimulation. We conclude that type I collagen may be involved in the progression of irreversible fibrosis. The imbalance between synthesis and degradation of extracellular matrix molecules or degree of stimulation over a certain period may lead to pancreatic fibrosis. Gene expressions of prolyl hydroxylase and tissue inhibitors of matrix metalloproteinase-2 were elevated.

Key words: Chronic pancreatitis, Fibrosis, Dibutyltin dichloride, WBN/Kob rat

Introduction

Chronic pancreatitis is histologically characterized by irregular fibrosis with destruction and loss of exocrine parenchyma, and these lesions are generally irreversible (Homma et al., 1997). Pancreatic fibrosis is classified into interlobular and intralobular types, and the implications and etiology of each type are well understood (Suda and Miyano, 1985; Suda et al., 1990, 1994, 1996, 2000). However, the cellular and molecular mechanisms leading to pancreatic fibrosis are not fully understood.

In order to clarify the mechanisms of pancreatic fibrosis, various experimental animal models have been utilized. For example, WBN/Kob rats (Tsuchitani et al., 1985; Ohashi et al., 1990), OLETF rats (Ishida et al., 1993) and Aly mice (Miyawaki et al., 1994) are known as spontaneously developing pancreatitis models, and cerulean-induced (Elsasser et al., 1992) or ethionine-induced (Rodriguez et al., 1994) pancreatitis are known as experimentally induced models. However, pancreatic fibrosis in animal models does not perfectly reflect human pancreatic fibrosis. Fibrosis of animal model is reversible, while that in human is irreversible. Better animal models for irreversible pancreatic fibrosis remain under development.

Nevertheless, it is possible to understand the pathogenesis of pancreatic fibrosis by investigating the differences in chronic pancreatitis between humans and animals. Some studies have demonstrated that higher levels of extracellular matrix molecules (ECM) are present in chronic pancreatitis. For example, both type I and type III collagens were detected in chronic pancreatitis in humans (Kennedy et al., 1987; Gress et al., 1994). We previously found that the fibrous area that decreased in WBN/Kob rats is mainly composed of type III collagen, which is relatively unstable when compared with type I collagen. In contrast, type I collagen was scarcely detectable. Thus, the transitional change in

fibrosis may be the result of production of type III collagen as a fibrotic component (Kakinuma et al., 1999).

Dibutyltin dichloride (DBTC) is used as a polyvinyl chloride stabilizer and catalyzer (Fent, 1996) and is known to cause chronic pancreatitis in rats (Merkord et al., 1997; Sparmann et al., 1997; Merkord et al., 1999). In this chronic pancreatitis model, the so-called DBTC model, epithelial damage and stenosis of the biliopancreatic duct initially occur, and subsequent chronic pancreatitis is induced. High levels of type I collagen are reported to be evident in the fibrous area and the pancreatic fibrosis in this model is apparently irreversible (Sparmann et al., 1997). Therefore, the present study was conducted to compare irreversible and reversible pancreatic fibrosis in 2 experimental animal models, *i.e.*, the irreversible DBTC model and the reversible spontaneous chronic pancreatitis in WBN/Kob rats.

There is considerable evidence that ECM components are regulated by both synthesis and degradation of matrix components (Gressner and Bachem, 1995). Prolyl hydroxylase (PH), composed of 2 types of subunit ($\alpha_2\beta_2$), is a key enzyme in the process of collagen biosynthesis (Cardinale and Udenfriend, 1974). Matrix metalloproteinases (MMPs) are enzymes that degrade both the collagenous and noncollagenous components of the ECM (Matrisian, 1990). MMP activities are inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs) (Matrisian, 1990). The expression of type III collagen, PH and TIMPs are not fully documented in experimental animal models. Therefore, we also investigated the expression of fibrosis-related genes in order to clarify the relationship between these genes and pancreatic fibrosis in the DBTC model.

In addition, we investigate the relationship between stimuli and fibrotic reactions in the pancreas. We thus prepared rats in which the bile duct is ligated at the duodenal portion (BDL model), and histopathologically compared the characteristics of pancreatic fibrosis with those from DBTC and BDL models.

Materials and methods

Animals and chemicals

Five-week-old LEW/Crj male rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan). Male and female WBN/Kob rats aged 4, 6 and 8 months were purchased from Japan SLC (Shizuoka, Japan). Seven-week-old Crj:CD(SD) IGS male rats were obtained from Charles River Japan, Inc. Animals were housed individually in cages and were maintained under standard laboratory conditions: room temperature, $23\pm 2^\circ\text{C}$; relative humidity, $55\pm 15\%$; 12-h/12-h light/dark cycle. Gamma ray-irradiated solid diets (CRF-1 for LEW/Crj rats and Crj:CD(SD) IGS rats, Charles River Japan; MB-3 for WBN/Kob rats, Funabashi Farm,

Chiba, Japan) and water were given *ad libitum*. DBTC was obtained from Wako Pure Chemical (Osaka, Japan), and was dissolved in 2 parts 100% ethanol and 3 parts glycerol.

Experimental procedure

In the DBTC model, male LEW/Crj rats were injected intravenously with a single dose of 8 mg/kg DBTC. In the control group, animals were treated with vehicle. General symptoms were observed daily and body weight was examined weekly in both groups during the experimental period. Five animals were sacrificed at each time point by exsanguination under pentobarbital sodium anesthesia (1 and 3 days, and 1, 2, 4, 8 and 24 weeks after treatment). Age-adapted WBN/Kob rats (5 males and 5 females at each of these ages) were obtained a week before sacrifice for acclimatization and were exsanguinated under pentobarbital sodium anesthesia. In BDL rats, bile duct ligation was performed in 9 rats under ether anesthesia. Using sterile techniques, bile ducts were ligated near the duodenum. Three rats receiving sham operation were subjected only to the manipulation of the bile duct, and served as controls. BDL rats were studied 3, 7 and 14 days after surgery, and sham rats were studied 3 day after surgery. The pancreas was removed quickly, and splenic, duodenal and colonic samples were fixed in 10% phosphate-buffered formalin. The remainder of the pancreas tissues in the DBTC model was stored in liquid nitrogen until analysis could be performed. Fixed tissues were processed for histological observation by conventional methods, stained with hematoxylin and eosin (H-E) or Masson's trichrome and examined histopathologically.

For immunohistochemical staining, antibodies against type I collagen (Biodesign International, Kennebunk, Me.) and type III collagen (Biodesign International) were used as primary antibodies. Deparaffinized sections were treated with 3% H_2O_2 in methanol for 15 min in order to inactivate endogenous peroxidase, rinsed 3 times in phosphate-buffered saline for 10 min each time, preincubated with normal goat serum (Dako, Golstrup, Denmark) for 30 min, and incubated for 30 min with a 1:300 dilution of primary antibody at room temperature. Localization of primary antibody was detected with biotinylated secondary antibody and streptavidin-biotin complex conjugated with horseradish peroxidase (LSAB2 Universal Kit, Dako). Sections were finally treated with the chromogen 3,3'-diaminobenzidine (DAB; 10 mg/ml, Sigma-Aldrich, St. Louis, Mo) substrate medium at room temperature for 10 min, counterstained with hematoxylin, and subjected to microscopic observation.

RT-PCR

Total RNA was extracted from pancreatic tissue by the acid guanidine isothiocyanate/phenol chloroform

method (Chomczynski and Sacchi, 1987). Following precipitation, RNA was resuspended in RNase-free TE buffer and concentration was estimated by absorbance at 260 nm. Samples were stored frozen at -80°C until analysis.

First-strand cDNA was synthesized from total RNA (2 mg) using an OmniscriptTM reverse transcriptase Kit (QIAGEN, Tokyo, Japan) with oligo(dT)₁₅ primer (Roche, Mannheim, Germany). PCR amplification for TGF- β 1, type I collagen, type III collagen, TIMP-2, and PH α - and β -subunit transcripts was performed with a KOD-Taq polymerase kit (TOYOBO, Osaka, Japan). The following primers were used: for type I collagen (Effah Kaufmann et al., 2000), sense 5'-CCA ATC TGG TTC CCT CCC AC-3' and antisense 5'-GTA AGG TTG AAT GCA CTT-3'; for type III collagen, sense 5'-GGT GAA ACT GGT GAA CGT GG-3' and antisense 5'-CAC CTC CAA CTC CAG CAA TG-3' (accession no. AJ005395); for TIMP-2 (Kim et al., 2000), sense 5'-AGA CGT AGT GAT CAG GGC CA-3' and antisense 5'-GTA CCA CGC GCA AGA ACC AT-3'; for PH α -subunit, sense 5'-TAC GAA ATG CTG TGC CGT GG-3' and antisense 5'-TAG CCA GAC AGC CAA GCA CT-3' (accession no. X78949); for PH β -subunit, sense 5'-TTC TGC TGT TCC TGC CCA AG-3' and antisense 5'-TGC TTG TCC CAG TCT TCA GG-3' (accession no. NM_012998); for cyclophilin (Danielson et al.,

1988), sense 5'-GGA TTC ATG TGC CAG GGT GG-3' and antisense 5'-CAC ATG CTT GCC ATC CAG CC-3'. PCR primers for rat TGF- β 1 were purchased (Clontech Laboratories, Palo Alto, CA). Cyclophilin was used as a reference gene for semiquantitative PCR. The optimal number of cycles for PCR of each primer was determined using serial dilutions of cDNA in PCR until a linear response was obtained. The optimal number of cycles was 33 for all cDNAs, except for cyclophilin (27 cycles). PCR parameters for all cDNAs were as follows: initial denaturation at 94°C for 2 min, followed by denaturation at 94°C for 20 s, annealing at 57°C for 45 s, and extension at 72°C for 45 s. PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide and visualized under UV light.

Results

Necropsy

In the DBTC model, there were no marked changes at day 1. Three of five rats exhibited dilation of the bile duct at 3 days after treatment. The duct was obstructed near the duodenal duct and filled with a yellowish secretion, becoming cystic at 1 week after treatment. These animals also exhibited yellowish ascites and systemic jaundice. This obstruction persisted during the

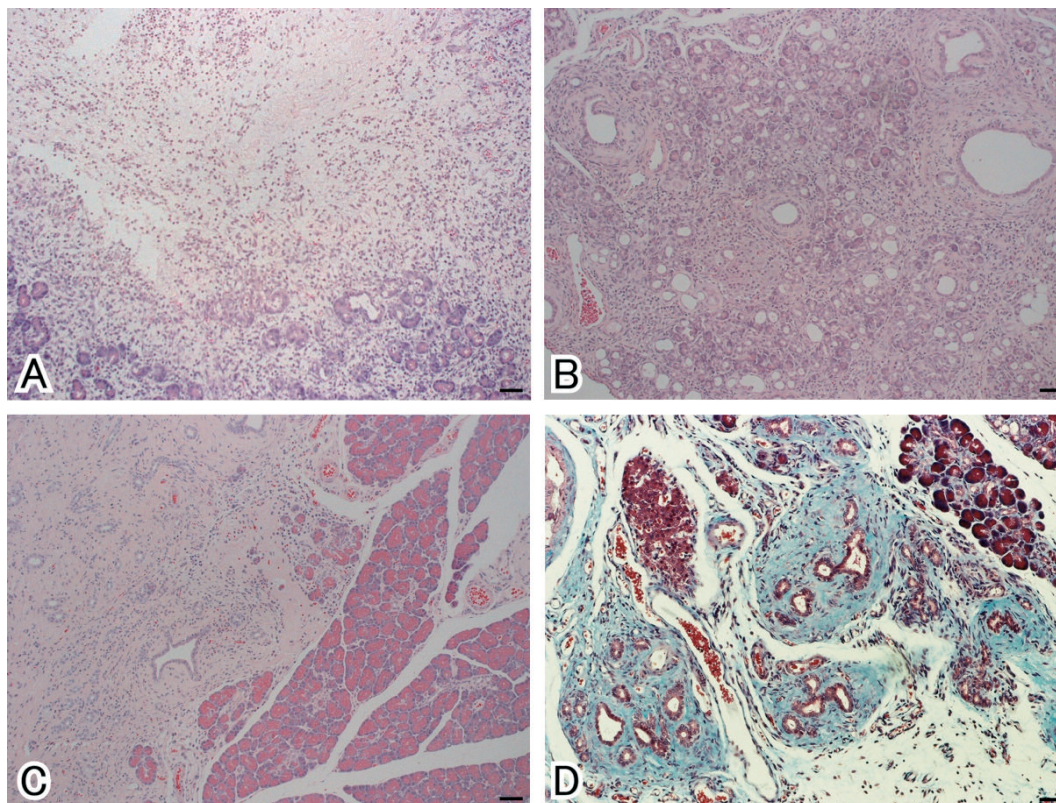


Fig. 1. Histopathological appearance of pancreas in rats treated with DBTC, after 3 days (A), 2 weeks (B), 8 weeks (D) and 24 weeks (C). A. H-E stain. Severe necrosis of epithelial cells from the pancreatic duct, edema and inflammatory cell infiltration are observed at 3 days after DBTC treatment. B. H-E stain. Inflammatory cell infiltration, fibrosis around the pancreatic duct, atrophy and necrosis of acinar cells are observed at 2 weeks after DBTC treatment. C. H-E stain. Fibrosis around the pancreatic duct is observed at 24 weeks after DBTC treatment. D. Masson's trichrome stain. Representative pancreatic sections taken from rats 8 weeks after DBTC treatment. The fibrous areas were mainly composed of collagen fibers. Bar: 50 μm .

experimental period. The BDL model also exhibited dilation of the bile duct at 3 days after treatment and cystic features at 1 week after treatment. All BDL rats died from 7 to 14 days after surgery. In contrast, the common bile duct was normal and jaundice was not observed in WBN/Kob rats in any point in the experimental period.

Histopathology

In the DBTC model at 3 days after treatment, edema, inflammatory cell infiltration, degeneration and necrosis of epithelial cells from the intralobular or interacinar pancreatic ducts and necrosis of periductal connective tissues were seen (Fig. 1A). After 1 and 2 weeks, dilation of the pancreatic ducts and fibrosis around the pancreatic ducts and intralobular areas were observed, while regenerative epithelial cells were scattered among the degenerated or necrotic epithelial cells (Fig. 1B). From 4 to 24 weeks after treatment, persistent fibrosis

was found around the pancreatic ducts and there was no trend toward regression (Fig. 1C). In contrast, interlobular fibrosis was partly formed but most of the intralobular fibrosis had regressed by that time. Masson's trichrome staining revealed that the fibrous areas were mainly composed of collagen fibers (Fig. 1D).

In contrast, pancreatic fibrosis in male WBN/Kob rats peaked at 4 months of age (Fig. 2A) and tended to regress by 8 months of age (Fig. 2B). Pancreatic fibrosis in these rats was mainly distributed in the intralobular regions and extended with division into some of the pancreatic islets (Fig. 2C). None of the animals showed protein plug or calculi in the ducts.

In the BDL model, massive necrosis sporadically occurred and pancreatic ducts were necrotic and desquamated epithelial cells, inflammatory cells or blood clots and the inflammatory area spread increasingly around the ducts was observed at 3 day after treatment (Fig. 3A,B). Fibrosis was observed

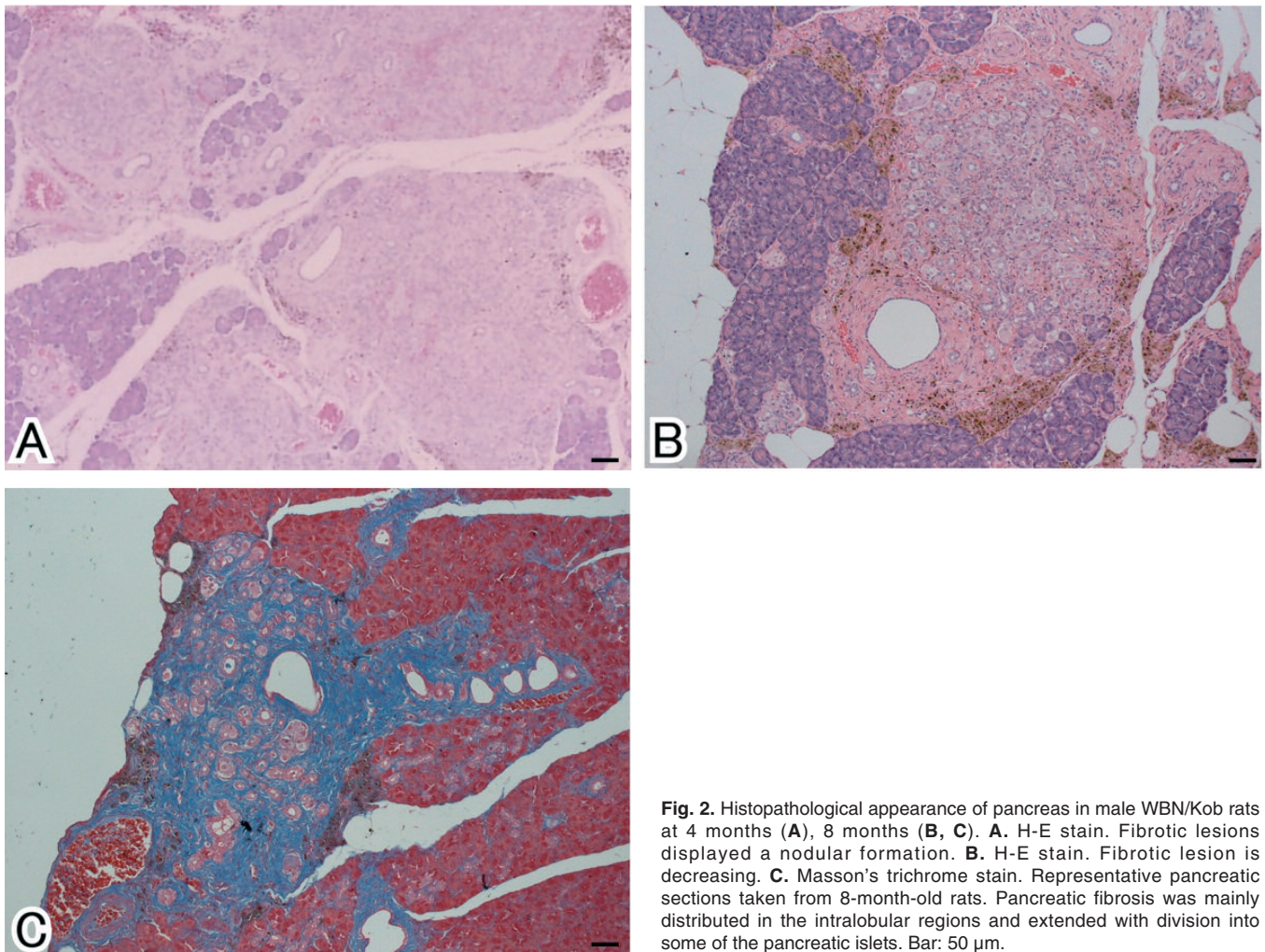


Fig. 2. Histopathological appearance of pancreas in male WBN/Kob rats at 4 months (A), 8 months (B, C). A. H-E stain. Fibrotic lesions displayed a nodular formation. B. H-E stain. Fibrotic lesion is decreasing. C. Masson's trichrome stain. Representative pancreatic sections taken from 8-month-old rats. Pancreatic fibrosis was mainly distributed in the intralobular regions and extended with division into some of the pancreatic islets. Bar: 50 μ m.

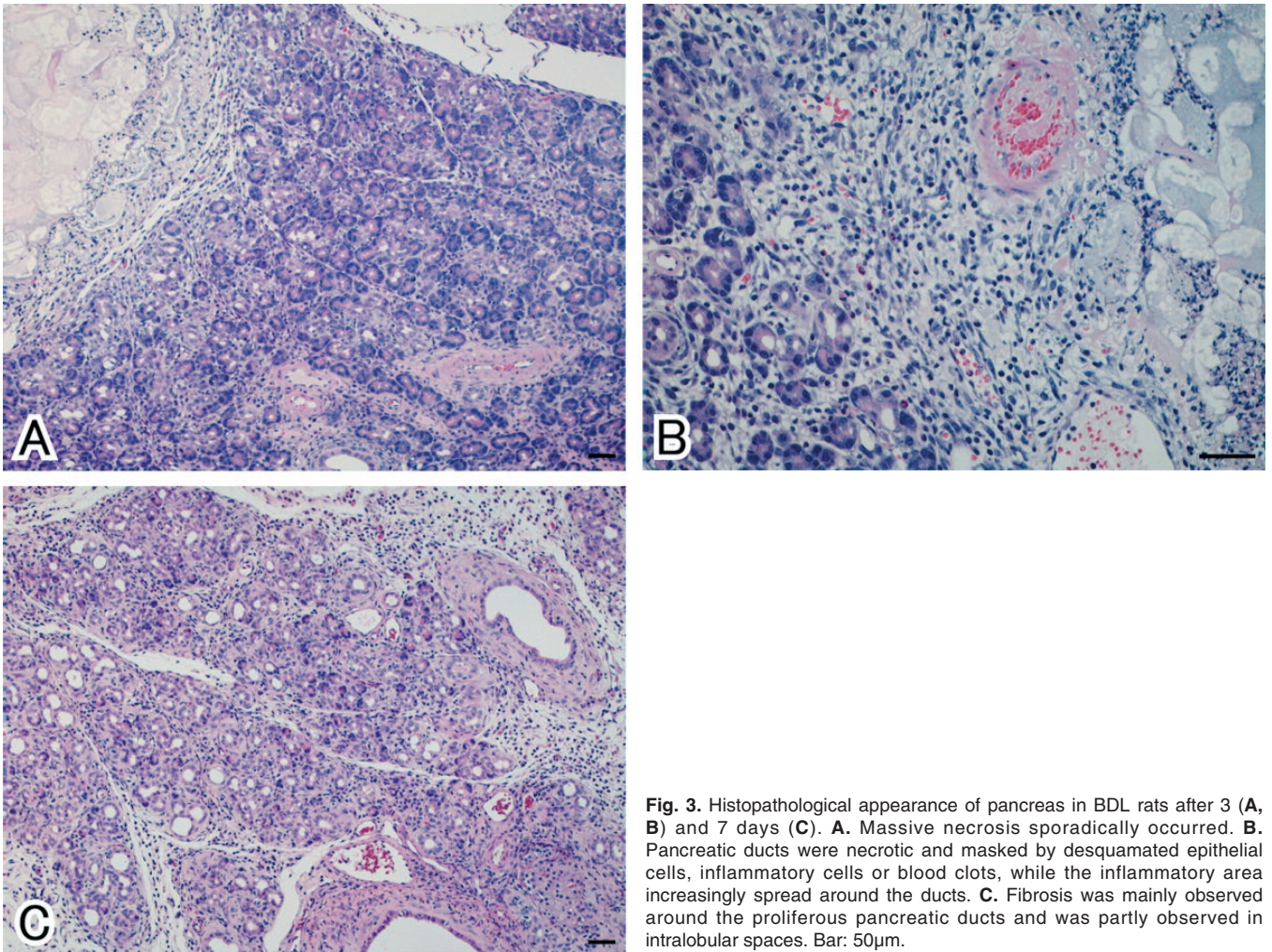


Fig. 3. Histopathological appearance of pancreas in BDL rats after 3 (A, B) and 7 days (C). A. Massive necrosis sporadically occurred. B. Pancreatic ducts were necrotic and masked by desquamated epithelial cells, inflammatory cells or blood clots, while the inflammatory area increasingly spread around the ducts. C. Fibrosis was mainly observed around the proliferous pancreatic ducts and was partly observed in intralobular spaces. Bar: 50µm.

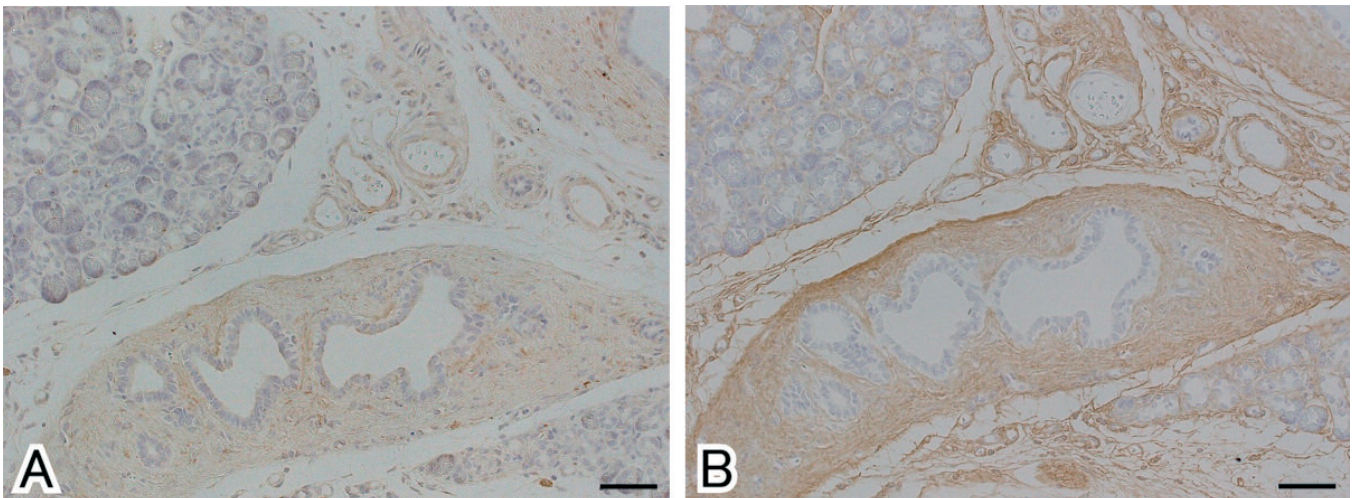


Fig. 4. Immunohistochemical localization of type I (A) and type III (B) collagen in DBTC-treated rats. Type I and type III collagen-positive cells are seen around the pancreatic duct at 24 weeks after DBTC treatment. Bar: 50µm.

around the proliferous pancreatic ducts and was partly observed at intralobular spaces at 7 days after treatment (Fig. 3C). Thus, the substantial fibrosis, which is known to occur in human biliary pancreatitis, was not observed in this study.

There were no significant changes in the pancreases of control LEW rats and female WBN/Kob rats throughout the experimental period. Sham operated rats in the BDL model also showed no pancreatic changes.

Immunohistochemistry

DBTC models had both type I and type III collagen in the fibrotic areas around the pancreatic ducts. Type III collagen was also observed in the larger areas spread around the periductal fibrotic area. At week 24, type I collagen was observed but was confined to the pancreatic duct (Fig. 4A,B).

On the other hand, WBN/Kob rats exhibited type III collagen in almost all fibrotic areas (Fig. 5A,B), while type I collagen was scarce at any age (Fig. 5C,D).

The BDL model also exhibited type III collagen around the pancreatic ducts. However type I collagen was not detected during our experimental period (data not shown).

RT-PCR

In the DBTC model, expression of fibrosis-related genes (TGF- β 1, type I and type III collagens, PH α and

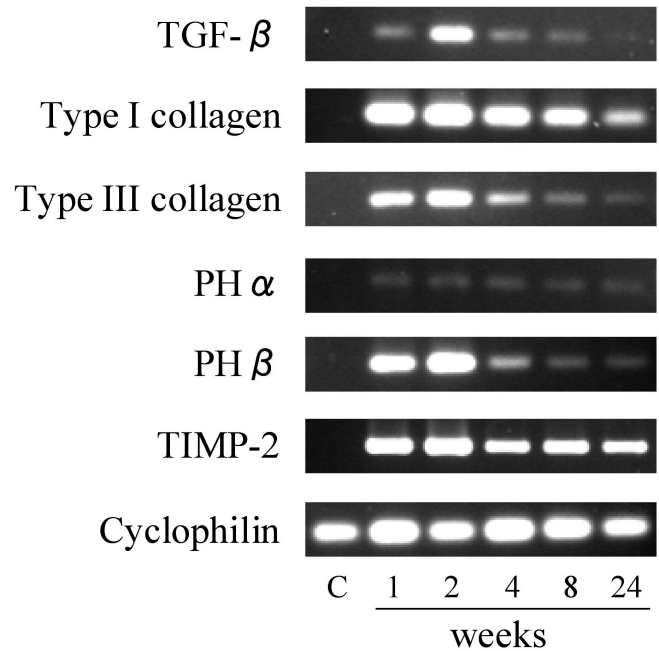


Fig. 6. Representative expression pattern of fibrosis-related genes in DBTC-treated rats.

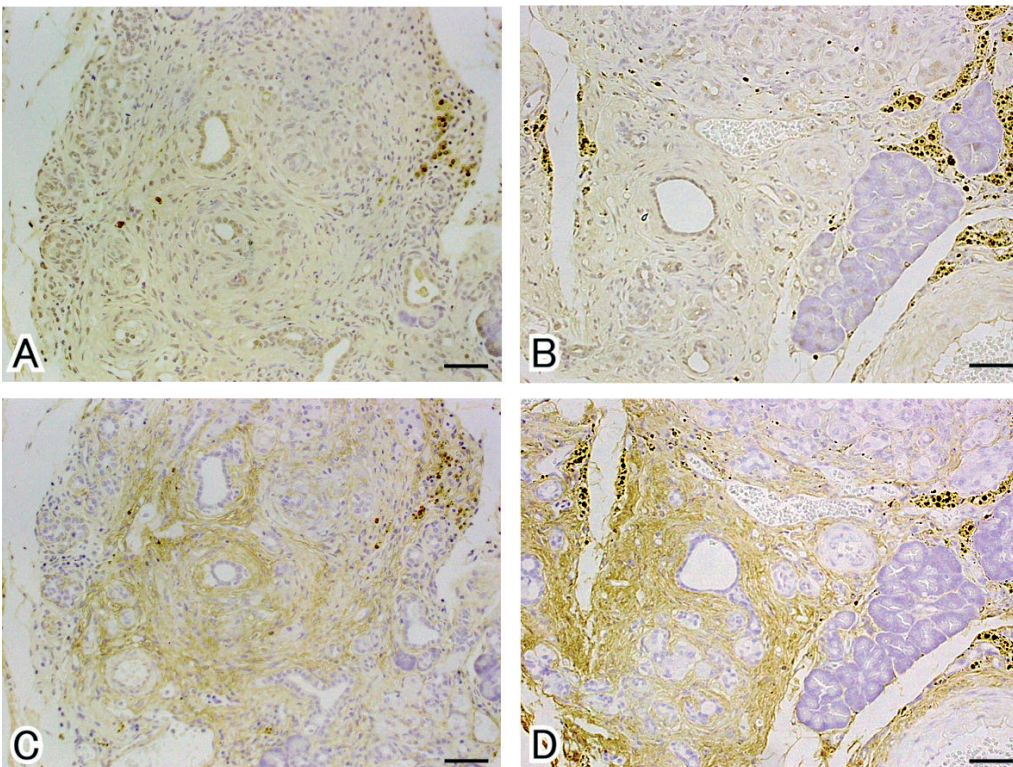


Fig. 5. Representative immunohistochemical localization of type I (A, B) and type III (C, D) collagen in male WBN/Kob rats at 4 months (A, C) and 8 months (B, D). Type III collagen is widespread in the fibrotic area in 4 and 8-month-old WBN/Kob rats. Type I collagen is scarce at any age. Bar: 50 μ m.

β subunits and TIMP-2) was assessed by RT-PCR (Fig. 6). Pancreatic tissue from normal LEW rats showed almost no expression of these fibrosis-related genes. However, expression of TGF- β 1 mRNA began to increase at 1 week after treatment, peaked at week 2, gradually decreased at weeks 4 and 8 and became diminished at week 24. Type I and type III collagen was markedly elevated at weeks 1 and 2 and gradually decreased until 24 weeks after treatment. The expression of type I collagen was apparently greater than that of type III collagen. Although the levels of PH α subunit mRNA were slightly elevated and became constant after DBTC treatment, those of β subunit mRNA were markedly elevated at weeks 1 and 2. TIMP-2 mRNA was elevated after DBTC treatment and this high level expression was maintained during the experimental period.

Discussion

The present study revealed that the area, components, progression and regression of pancreatic fibrosis in the DBTC model differ from those in WBN/Kob rats. Specifically, pancreatic fibrosis in the DBTC model mainly involved the appearance of type I collagen around the pancreatic duct and regression of fibrosis in that area was weak when compared with the intralobular area. In contrast, pancreatic fibrosis in male WBN/Kob rats was mainly observed in the intralobular area. Although fibrosis in male WBN/Kob rats was also seen around the pancreatic duct at the time of marked fibrosis, type I collagen was scarcely expressed there, and these animals exhibited a tendency for regression in all fibrous areas when compared with the DBTC model. These observations thus indicate that the expression of type I collagen is important for the irreversibility of fibrosis. This hypothesis is also supported by the fact that fibrosis remained only in the areas in which long-term expression of type I collagen was seen.

In humans, pancreatic fibrosis is classified into interlobular and intralobular types (Suda and Miyano, 1985; Suda et al., 1990, 1994, 1996, 2000). In chronic alcoholic pancreatitis, fibrosis is mainly found in the interlobular or perilobular areas, whereas in patients with alcoholic dependence syndrome, fibrosis is distributed mainly in the intralobular areas. Immunohistochemical analysis has shown that perilobular fibrosis is positive for types I and III collagen, whereas intralobular fibrosis is positive for type III collagen (Suda et al., 1993). Type I collagen is the major fibrillar collagen, and has high tensile strength and limited elasticity (Henkel and Glanville, 1982; Keene et al., 1987). Type III collagen is another major fibrillar collagen, and its structure and arrangement are similar to those of type I collagen (Henkel and Glanville, 1982; Keene et al., 1987). However, it forms thinner and more elastic fibers (Henkel and Glanville, 1982; Keene et al., 1987). Type III collagen is coordinately expressed with type I collagen (Ruteshouser and de Crombrughe, 1989). It is

thought that fibrosis occurring after acute pancreatitis involves type III collagen, which is ultimately replaced by irreversible type I collagen for the duration of the disease (Suda and Tsukahara, 1992). Type III collagen is relatively unstable in comparison to type I collagen, and deposition of type III collagen or fibronectin is largely reversible (Kennedy et al., 1984). Kakinuma et al. reported that pancreatic fibrosis in male WBN/Kob rats probably disappeared as the fibrous components consist mainly of reversible type III collagen (Kakinuma et al., 1999). The present comparative study in 2 pancreatitis models confirmed that type I collagen may be involved in the progression of irreversible fibrosis.

The results of RT-PCR in DBTC model revealed that the mRNA levels of PH α - and β -subunits are elevated in pancreatic fibrosis and that levels of the type I collagen transcript increased in parallel with those of the TGF- β 1 transcript.

There is considerable evidence that both the synthesis and degradation of ECM components are regulated (Gressner and Bachem, 1995). PH is a key enzyme that hydroxylates peptide-bound proline during collagen biosynthesis (Cardinale and Udenfriend, 1974). TIMP-2 has a dual action on MMP-2; it binds to MMP-2 and inhibits the activity of MMP-2 (Gress et al., 1994). TIMP-2 is also an important activator of proMMP-2. Once secreted, proMMP-2 and TIMP-2 bind to the cell surface, where proMMP-2 is activated by tri-molecular complex formation with MT1-MMP (Itoh et al., 2001). However, in the presence of excess TIMP-2, MT1-MMP-2 is inhibited and MMP-2 is not activated (Shek et al., 2002). To date, the sequential changes in the activities of these genes during the onset and progression of pancreatic fibrosis have remained unclear. The expression of TIMP-2 is also elevated. Our results also indicate that overexpression of TIMP-2 may promote imbalance between the synthesis and degradation of the ECM and may be a pathogenic factor leading to pancreatic fibrosis and the result of the TGF- β 1 showed that the transcription of TGF- β 1 is also involved in our experimental fibrosis from DBTC.

TGF- β is known to play a crucial role in fibrosis by increasing the production of ECM and decreasing the degradation of ECM components. Part of the action of TGF- β on gene expression is exerted at the transcriptional level. For example, a strong TGF- β response element was identified within the 3.5-kb upstream region of the human $\alpha_2(I)$ collagen gene (Inagaki et al., 1994). It has also been reported that TGF- β stimulates the human $\alpha_2(I)$ collagen gene by increasing the affinity of an Sp1-containing transcriptional complex for an upstream TGF- β response element (Inagaki et al., 1994).

We also contrasted the features of the DBTC model with those of the BDL model. The aim was to investigate the relationship between the degree of stimulation and fibrous features. DBTC causes toxic necrosis of the biliopancreatic duct epithelium in rats, leading to duct obstruction and interstitial pancreatitis

followed by periductal and interstitial fibrosis. In the present study, necrosis of the biliopancreatic duct and duct obstruction were observed, particularly in the duodenal part of the gland. The bile ducts of rats are combined in the duodenal region, forming the common pancreatic duct. In the DBTC model, we observed high levels of amylase and lipase in bile (data not shown). We speculate that due to the anatomical characteristics of the biliopancreatic duct in rats, bile regurgitation may occur subsequent to obstruction of the pancreatic duct by DBTC. Fibrosis in biliary pancreatitis is mainly observed around the pancreatic duct, and this fibrosis extends to interlobular areas.

One factor involved in the pathogenesis of biliary pancreatitis is thought to be abnormal pancreatic choledochoductal junction or pancreaticobiliary maljunction (PBM). If the DBTC or BDL model is a model for biliary pancreatitis in anomalous arrangement of the pancreaticobiliary ducts, our results suggest that progression of fibrosis requires a continual stimulus. In other words, the DBTC model exhibited persistent fibrosis around the pancreatic duct and was comparatively similar in histology to fibrosis from biliary pancreatitis. The BDL model exhibited spacious necrosis and severe proliferation of the pancreatic duct and showed comparatively marked fibrosis at the intralobular space. In addition, there was a lower tendency to develop interlobular fibrosis. We believe that these differences are based on the variety of stimuli.

The pancreatic lesions in both models were induced by constriction of the biliopancreatic duct adjacent to the duodenum. However, the degree of constriction in each model was obviously different, i.e., physical ligation ensures perfect stenosis. Thus, it is possible that the formation of persistent fibrosis at the periductal or interlobular area in the DBTC model was the result of comparatively weak stimulation. Although the process of fibrosis in the DBTC model resembles that in biliary pancreatitis, biliary pancreatitis is not likely to develop into chronic pancreatitis (Suda and Miyano, 1985; Kloppel and Maillet, 1992). In order to develop a suitable model for pancreatic fibrosis in human biliary pancreatitis based on pancreaticobiliary abnormalities, it thus appears necessary to devise methods for providing stimulation of the pancreatic ducts.

In conclusion, the present study confirmed that type I collagen may be involved in the progression of irreversible fibrosis. The imbalance between synthesis and degradation of the ECM or degree of stimulation during certain periods may be a pathogenic factor leading to pancreatic fibrosis. Further investigation is needed in order to elucidate the precise mechanisms underlying the change from type III collagen to type I collagen that occurs in chronic pancreatitis models.

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References

- Cardinale G.J. and Udenfriend S. (1974). Prolyl hydroxylase. *Adv. Enzymol. Relat. Areas Mol. Biol.* 41, 245-300.
- Chomczynski P. and Sacchi N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162, 156-159.
- Danielson P.E., Forss-Petter S., Brow M.A., Calavetta L., Douglass J., Milner R.J. and Sutcliffe J.G. (1988). p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. *DNA* 7, 261-267.
- Effah Kaufmann E.A., Ducheyne P. and Shapiro I.M. (2000). Evaluation of osteoblast response to porous bioactive glass (45S5) substrates by RT-PCR analysis. *Tissue Eng.* 6, 19-28.
- Elsasser H.P., Haake T., Grimmig M., Adler G. and Kern H.F. (1992). Repetitive cerulein-induced pancreatitis and pancreatic fibrosis in the rat. *Pancreas* 7, 385-390.
- Fent K. (1996). Ecotoxicology of organotin compounds. *Crit. Rev. Toxicol.* 26, 1-117.
- Gress T.M., Muller-Pillasch F., Lerch M.M., Friess H., Buchler M., Beger H.G. and Adler G. (1994). Balance of expression of genes coding for extracellular matrix proteins and extracellular matrix degrading proteases in chronic pancreatitis. *Z. Gastroenterol.* 32, 221-225.
- Gressner A.M. and Bachem M.G. (1995). Molecular mechanisms of liver fibrogenesis—a homage to the role of activated fat-storing cells. *Digestion* 56, 335-346.
- Henkel W. and Glanville R.W. (1982). Covalent crosslinking between molecules of type I and type III collagen. The involvement of the N-terminal, nonhelical regions of the alpha 1 (I) and alpha 1 (III) chains in the formation of intermolecular crosslinks. *Eur. J. Biochem.* 122, 205-213.
- Homma T., Harada H. and Koizumi M. (1997). Diagnostic criteria for chronic pancreatitis by the Japan Pancreas Society. *Pancreas* 15, 14-15.
- Inagaki Y., Truter S. and Ramirez F. (1994). Transforming growth factor-beta stimulates alpha 2(I) collagen gene expression through a cis-acting element that contains an Sp1-binding site. *J. Biol. Chem.* 269, 14828-14834.
- Ishida K., Mizuno A., Sano T., Shi K. and Shima K. (1993). Plasma glucagon responses to insulin-induced hypoglycemia and arginine in spontaneous non-insulin-dependent diabetes mellitus (NIDDM) rats, Otsuka Long Evans Tokushima Fatty (OLETF) strain. *Acta. Endocrinol. (Copenh)* 129, 585-593.
- Itoh Y., Takamura A., Ito N., Maru Y., Sato H., Suenaga N., Aoki T. and Seiki M. (2001). Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion. *EMBO J.* 20, 4782-4793.
- Kakinuma C., Suda K. and Shibutani Y. (1999). Histopathological and biochemical studies on pancreatic fibrosis in WBN/Kob rats. *Virchows Arch.* 434, 83-89.
- Keene D.R., Sakai L.Y., Bachinger H.P. and Burgeson R.E. (1987). Type III collagen can be present on banded collagen fibrils regardless of fibril diameter. *J. Cell Biol.* 105, 2393-2402.
- Kennedy R.H., Uscanga L., Choux R. and Grimaud J.A. (1984). Pancreatic connective matrix changes in acute pancreatitis. In: *Pancreatitis: concepts and classification*. Gyr K.E., Singer M.V. and Sarles H. (eds). Elsevier Science. Amsterdam. pp 147-148.
- Kennedy R.H., Bockman D.E., Uscanga L., Choux R., Grimaud J.A. and Sarles H. (1987). Pancreatic extracellular matrix alterations in chronic pancreatitis. *Pancreas* 2, 61-72.

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- Kim M.H., Albertsson P., Xue Y., Kitson R.P., Nannmark U. and Goldfarb R.H. (2000). Expression of matrix metalloproteinases and their inhibitors by rat NK cells: inhibition of their expression by genistein. *In Vivo* 14, 557-564.
- Kloppel G. and Mailliet B. (1992). The morphological basis for the evolution of acute pancreatitis into chronic pancreatitis. *Virchows Arch. (A)*. 420, 1-4.
- Matrisian L.M. (1990). Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet.* 6, 121-125.
- Merkord J., Jonas L., Weber H., Kroning G., Nizze H. and Hennighausen G. (1997). Acute interstitial pancreatitis in rats induced by dibutyltin dichloride (DBTC): pathogenesis and natural course of lesions. *Pancreas* 15, 392-401.
- Merkord J., Weber H., Sparmann G., Jonas L. and Hennighausen G. (1999). The course of pancreatic fibrosis induced by dibutyltin dichloride (DBTC). *Ann. NY Acad. Sci.* 880, 231-237.
- Miyawaki S., Nakamura Y., Suzuka H., Koba M., Yasumizu R., Ikehara S. and Shibata Y. (1994). A new mutation, *aly*, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. *Eur. J. Immunol.* 24, 429-434.
- Ohashi K., Kim J.H., Hara H., Aso R., Akimoto T. and Nakama K. (1990). WBN/Kob rats. A new spontaneously occurring model of chronic pancreatitis. *Int. J. Pancreatol.* 6, 231-247.
- Rodriguez A.I., de Dios I., Garcia-Montero A.C. and Manso M.A. (1994). Acute necrotizing pancreatitis induced by a CDE diet in the rat: a morphologic and functional study. *J. Lab. Clin. Med.* 123, 693-700.
- Ruteshouser E.C. and de Crombrughe B. (1989). Characterization of two distinct positive cis-acting elements in the mouse alpha 1 (III) collagen promoter. *J. Biol. Chem.* 264, 13740-13744.
- Shek F.W., Benyon R.C., Walker F.M., McCrudden P.R., Pender S.L., Williams E.J., Johnson P.A., Johnson C.D., Bateman A.C., Fine D.R. and Iredale J.P. (2002). Expression of transforming growth factor-beta 1 by pancreatic stellate cells and its implications for matrix secretion and turnover in chronic pancreatitis. *Am. J. Pathol.* 160, 1787-1798.
- Sparmann G., Merkord J., Jaschke A., Nizze H., Jonas L., Lohr M., Liebe S. and Emmrich J. (1997). Pancreatic fibrosis in experimental pancreatitis induced by dibutyltin dichloride. *Gastroenterology* 112, 1664-1672.
- Suda K. and Miyano T. (1985). Bile pancreatitis. *Arch. Pathol. Lab. Med.* 109, 433-436.
- Suda K., Mogaki M., Oyama T. and Matsumoto Y. (1990). Histopathologic and immunohistochemical studies on alcoholic pancreatitis and chronic obstructive pancreatitis: special emphasis on ductal obstruction and genesis of pancreatitis. *Am. J. Gastroenterol.* 85, 271-276.
- Suda K. and Tsukahara M. (1992). Histopathological and immunohistochemical studies on apparently uninvolved areas of pancreas in patients with acute pancreatitis. *Arch. Pathol. Lab. Med.* 116, 934-937.
- Suda K., Akai J. and Nakamura T. (1993). Pancreatic fibrosis in patients with alcoholic dependence syndrome. *Arch. Pathol. Lab. Med.* 117, 1013-1016.
- Suda K., Shiotsu H., Nakamura T. and Akai J. (1994). Pancreatic fibrosis in patients with chronic alcohol abuse: correlation with alcoholic pancreatitis. *Am. J. Gastroenterol.* 89, 2060-2062.
- Suda K., Takase M., Takei K., Nakamura T. and Akai J. (1996). Histopathologic study of coexistent pathologic states in pancreatic fibrosis in patients with chronic alcohol abuse: two distinct pathologic fibrosis entities with different mechanisms. *Pancreas* 12, 369-372.
- Suda K., Takase M., Takei K., Kumasaka T. and Suzuki F. (2000). Histopathologic and immunohistochemical studies on the mechanism of interlobular fibrosis of the pancreas. *Arch. Pathol. Lab. Med.* 124, 1302-1305.
- Tsuchitani M., Saegusa T., Narama I., Nishikawa T. and Gonda T. (1985). A new diabetic strain of rat (WBN/Kob). *Lab. Anim.* 19, 200-207.

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