Virchows Arch (1999) 434:83–89

ORIGINAL ARTICLE

Chihaya Kakinuma · Koichi Suda
Yasunori Shibutani

Histopathological and biochemical studies on pancreatic fibrosis in WBN/Kob rats

Received: 1 October 1998 / Accepted: 2 October 1998

Abstract We investigated the time-course of changes in pancreatic fibrosis accompanied with pancreatitis in WBN/Kob rats. The areas of fibrosis and fatty replacement were analysed morphometrically, and biochemical measurements of pancreatic and plasma prolyl hydroxylase and of pancreatic collagenase were assessed. Male rats showed acute pancreatitis at 2–3 months of age, lesions that later underwent a transition to widespread fibrosis. The fibrosis then decreased, and the fibrotic tissue was replaced with adipose tissue. Morphometrically, the fibrotic area reached its maximal size when the rats were 4 months old, diminishing thereafter. The fibrosis occurred mainly in the intralobular space, and was principally attributable to type-III collagen. Type-I collagen scarcely appeared throughout the experimental period. α-Smooth muscle actin appeared in and around myofibroblasts that developed in an early stage and diminished later in accordance with the progressive manner of fibrosis. The plasma prolyl hydroxylase level was higher in males than in females from 4 through 10 months of age. Pancreatic collagenase activity in the males also increased during the same period. These findings suggest that pancreatic fibrosis in male WBN/Kob rats is affected by the balance between prolyl hydroxylase and collagenase.

Key words WBN/Kob rat · Pancreatitis · Prolyl hydroxylase · Collagenase

Introduction

Pancreatic fibrosis in patients with alcoholism or cholelithiasis develops during the progression of acute pancreatitis into chronic pancreatitis [2, 7, 9, 10]. Recently, pancreatic fibrosis has been classified into interlobular, intralobular, or other types according to its histological features; and the implications of this classification have become appreciated and the aetiology for each type has gradually been elucidated [18, 19, 20].

WBN/Kob rats [15, 23], OLETF rats [4], and Aly mice [14] are known to develop pancreatitis spontaneously, and experimental pancreatitis can be induced by ethanol feeding [24] or arginine injection [6] in rats or mice. These conditions have thus been utilized as models for human pancreatitis.

Pancreatic fibrosis in animal models is transient, decreasing noticeably sometime after development; thus, pancreatic fibrosis in animal models seems to be different from that in human chronic pancreatitis, which means it is difficult to extrapolate the mechanism for development of pancreatic fibrosis in animal models to that in humans. However, elucidation of the mechanism responsible for this decrease in fibrosis in animal models may help to develop future therapy for human pancreatic fibrosis. Recently, enzymes involved in the synthesis or degradation of collagen fibres have been analysed, and the mechanism of development of pancreatic fibrosis and its progressive decline has been partially disclosed in animal models [5, 24].

We examined male WBN/Kob rats by pathological and biochemical techniques. Pancreatitis occurs spontaneously after 2–3 months of age in these animals' and we used them as a model for pancreatic fibrosis with the aim of clarifying the mechanisms responsible for the later progressive decrease in fibrosis.

C. Kakinuma · K. Suda
Department of Pathology, Juntendo University,
School of Medicine, 2-1-1 Hongo, Bunkyo-ku,
Tokyo 113–8421, Japan

C. Kakinuma (✉) · Y. Shibutani
Toxicology Laboratory, Mochida Pharmaceutical Co., Ltd.,
342 Gensuke, Fujieda, Shizuoka 426–8640, Japan
Tel.: 81-54-636-7035, Fax: 81-54-636-2447

Materials and methods

WBN/Kob rats were purchased from Japan SLC (Shizuoka, Japan) and bred at the Toxicology Laboratory of Mochida Pharmaceutical Co. (Tokyo, Japan). The animals were maintained in an animal room with a controlled temperature (23±2°C) and humidity (55±15%) and a 12-h light/12-h dark cycle and were given a gamma ray-irradiated solid diet (MB-3; Funabashi Farm, Chiba, Ja-

pan) and water ad libitum during the experimental period. The guidelines of Mochida Pharmaceutical Co. for the care and use of animals were followed in this study. Animals aged 1, 2, 3, 4, 8, and 10 months (5 males and 5 females at each of these ages) were used in this study. Heparinized blood samples were collected from the animals under pentobarbital sodium anaesthesia. Subsequently, each animal was killed by exsanguination, and the pancreas was excised.

The pancreas was divided into the splenic, duodenal, and colonic parts, and fixed in 10% phosphate-buffered formalin. The tissues were embedded in paraffin, sliced into 3-µm sections, and stained with haematoxylin and eosin, Masson's trichrome, silver or Elastica-van Gieson's stain, and then subjected to histopathological and morphometric examination.

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

Using sections from each of five males at 2, 4, 8, and 10 months of age, the total pancreatic area including splenic, duodenal, and colonic parts and the areas of fibrosis and fatty replacement in them were determined morphometrically with an image analyser (LUSEX III; Nireco, Tokyo, Japan). The areas of fibrosis and fatty replacement were determined as proportions (%) of the total pancreatic area.

The concentration of amylase in plasma from each of five males at 2, 4, 8, and 10 months of age was measured enzymatically with an autoanalyser (Cobas Fara, Roche, Basel, Switzerland). The concentrations of prolyl hydroxylase in plasma and pancreatic tissue and the collagenase activities in pancreatic tissue from each of five males and five females at 2, 4, 8, and 10 months of age were determined by the methods described below.

Heparinized blood samples were centrifuged at 2,000 *g* for 15 min at 4°C to isolate plasma for determination of prolyl hydroxylase concentrations. The weighed pancreatic tissue was homogenized in 9 volumes of 10 mM Tris-HCl solution (9.25 M sucrose, 100 µM dithiothreitol, 10 µM EDTA, pH 7.4) with a Wahring blender (ULTRA-TURRAX, type T-25; Junke and Kunkel, Staufen, Germany). The homogenate was centrifuged at 5,000 *g* for 30 min at 4°C to isolate the supernatant fluid, which served as a sample for determination of prolyl hydroxylase protein levels. Prolyl hydroxylase concentrations were determined according to the methods of Yamada et al. [24] and Kodama et al. [11] by means of a rat prolyl hydroxylase-measurement kit (Fujiyakuhin Kogyo, Toyama, Japan). The concentration (µg) of prolyl hydroxylase was expressed as micrograms per milligram of protein. Protein concentrations were determined by the method of Lowry et al. [12], with bovine serum albumin used as a standard.

For determination of collagenase activity in the pancreas, the weighed pancreatic tissue was homogenized in 9 volumes of 50 mM Tris-HCl solution (0.2 M NaCl, 5 mM $CaCl_2$; pH 7.8) with a Wahring blender (ULTRA-TURRAX, type T-25). Next, the homogenate was diluted with 2 volumes of 50 mM Tris-HCl solution and incubated at 37°C for 2 h. Collagenase activity was assayed by use of fluorescein isothiocyanate-labelled collagen as a substrate (Collagenokit, CLN-100; Collagen Research Centre, Tokyo, Japan). Then, the collagenase activity (units) per gram of the pancreas was calculated.

Numerical data were expressed as the mean±standard deviation. Dunnett's *t*-test was used to analyse statistically significant differences between 2-month-old animals and 4-, 8-, or 10-month-old ones. For comparison between the males and females Student's *t*-test was used, and $P<0.05$ was considered significant.

Results

Female WBN/Kob rats showed no changes in the pancreas throughout the experimental period. Male rats also showed no changes until 1 month of age (Fig. 1A). At 2 months of age, some male rats showed interlobular haemorrhage, oedema, and inflammatory cell infiltration (Fig. 1B). At 3 months of age, haemorrhage, oedema, and inflammatory cell infiltration were observed in all male rats. The animals with severe inflammatory changes also showed degeneration and necrosis of acinar cells, proliferation of pancreatic ductules, and fibrosis to some degree (Fig. 1C). By 4 months of age, haemorrhage and oedema had decreased, and the sites of these lesions had become fibrotic. Most fibrotic areas were nodular, located in the lobules, and included atrophic islets and proliferated ducts (Figs. 1D, 2 A). Interlobular fibrosis was also seen in connection with the intralobular fibrosis. None of the animals at any age showed protein plugs or calculi in the ducts. In addition, neither necrosis of peripancreatic fatty tissue nor pseudocystic formation was observed throughout the experimental period. Masson's trichrome method and Elastica-van Gieson's stain revealed that the fibrotic areas in male rats were mainly composed of collagen fibres (data not shown). Silver-stained sections indicated scarce reticulum fibres in the fibrotic areas (Fig. 2B). At 8 months of age, the fibrotic area decreased, and the adipose tissue increased in area (Fig. 1E). By 10 months of age, the fibrotic area had continued to become smaller, and the area of fatty replacement had become even larger (Fig. 1F). There were no conspicious differences among the splenic, duodenal, and colonic parts in the occurrence or degree of these lesions.

Immunohistochemical examination showed that collagen fibres in the intralobular space were those of type-III collagen (Fig. 2C). Type-I collagen was very rare (Fig. 2D) and was located interlobularly. These findings on relating to type-I and type-III collagens were commonly obtained in tissue from male rats at 4 months of age or older. In addition, α-smooth muscle actin was observed in connection with the expansion of the fibrotic area. At 3–4 months of age, that is to say in the early stage of fibrotic expansion, an amorphous reaction for α-smooth muscle actin was recognized diffusely in the fibrotic areas of pancreas from male rats (Fig. 2E). In contrast, the region positive for (α-smooth muscle actin became linear in the fibrotic area and was located mainly around the vessels, pancreatic ducts, and islets of animals at 8 months of age or older (Fig. 2F).

Fibrotic area per total pancreatic area in male rats was maximal (20.0±5.7%) at 4 months of age. Thereafter, the fibrotic area decreased with increasing age: at 8 and 10 months of age the figures were 8.7±3.1% and 7.6±2.9%,

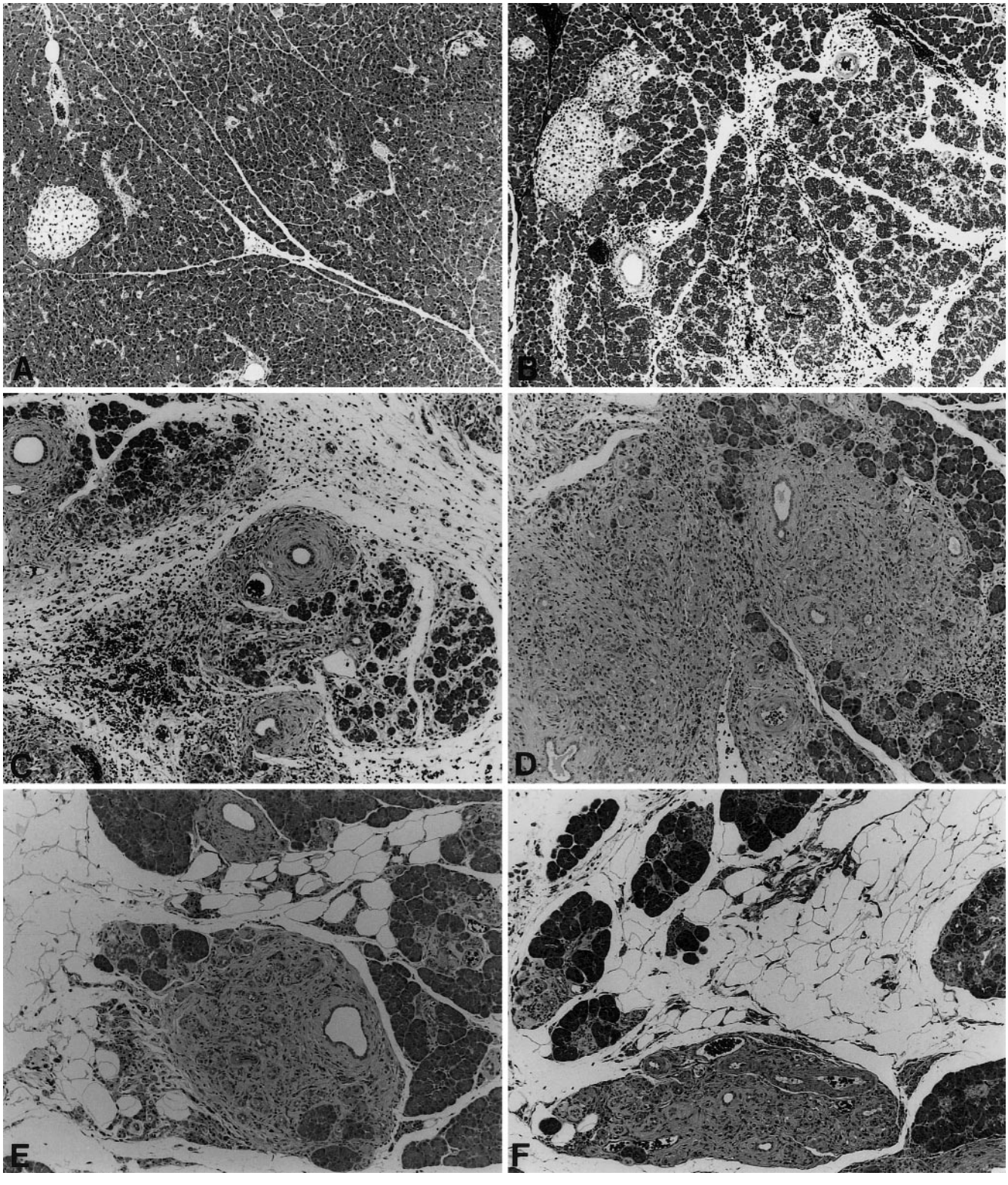

Fig. 1 **A–F** Histological features of the time-course of changes in pancreatitis of male WBN/Kob rats. Haematoxylin-eosin, ×80 **A** At 1 month of age, no abnormalities are seen. **B** At 2 months of age, haemorrhage and oedema are seen in the interlobular and intralobular spaces. **C** At 3 months of age, inflammation is widespread and accompanied by atrophy of acinar cells. **D** At 4 months of age, the fibrotic area expands and forms nodular style. Haemorrhage and oedema are becoming weaker. **E** At 8 months of age, the fibrotic area is decreasing and adipose tissue replacing it. Haemosiderin deposition is seen at the periphery of the fibrotic area. **F** At 10 months of age, areas of fibrosis and acinar cells are decreasing. Adipose tissue is widespread

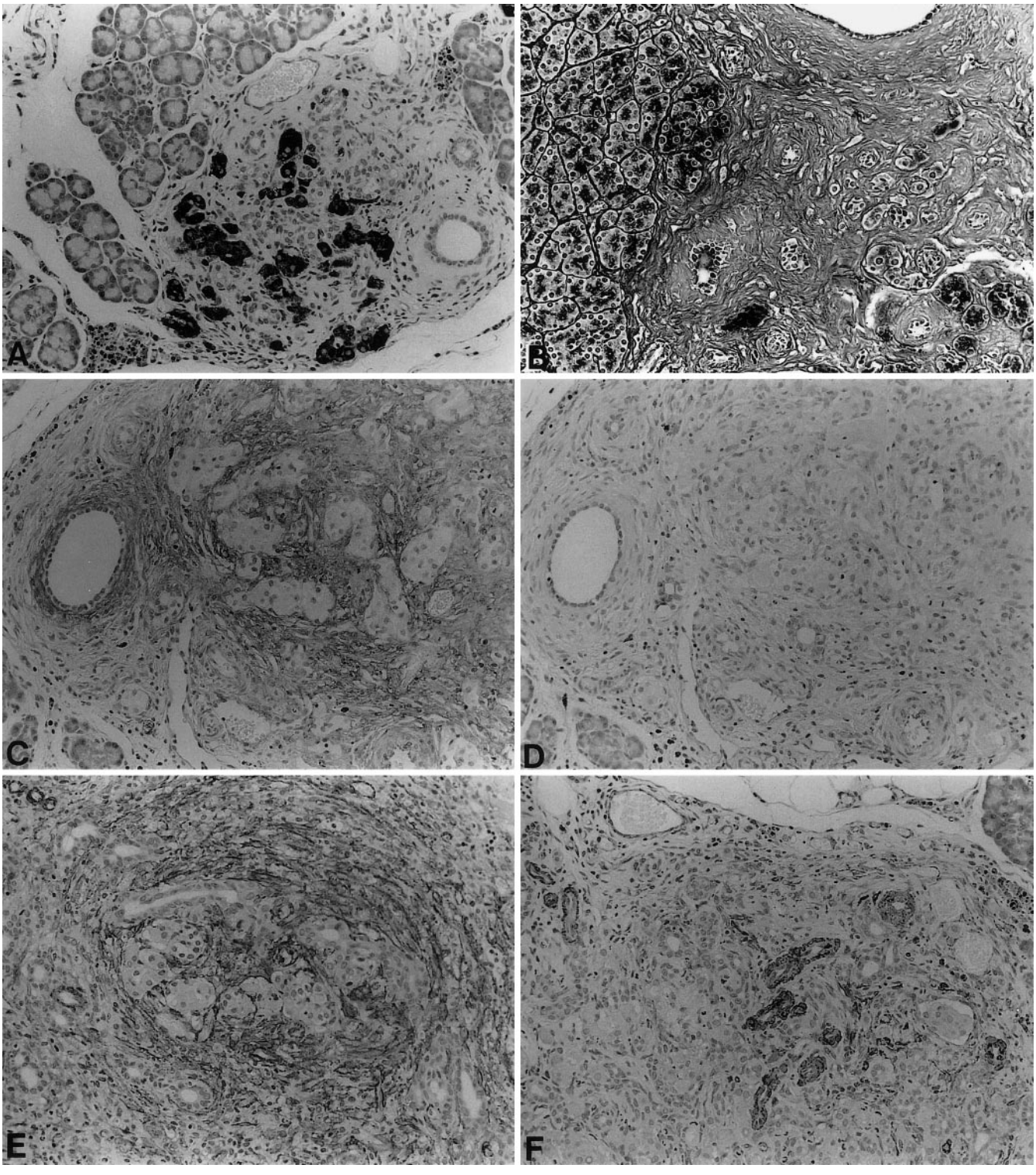

Fig. 2 **A–F** Characterization of the pancreatic fibrosis of male WBN/Kob rats. ×160 **A**, At 4 months of age, insulin-positive cells are scattered in the fibrotic area. Pancreatic ducts are also recognized. Insulin immunostaining **B** Reticulin fibres are seen at the basement membrane of acinar cells from a 4-month-old rat, whereas the fibres are very rare in the fibrotic area. The fibrotic area is mainly composed of collagen fibres, which are brownish-coloured. Silver stain **C** Type-III collagen is widespread in the fibrotic area at 4 months of age. Type-III collagen immunostaining **D** Type-I collagen is not detectable in the same area as shown in **C**. Type I-collagen immunostaining **E** α-Smooth muscle actin is widely observed in the fibrotic area at 4 months of age. α-Smooth muscle actin immunostaining **F** α-Smooth muscle actin is located exclusively around the pancreatic ducts and vessels at 8 months of age. α-Smooth muscle actin immunostaining

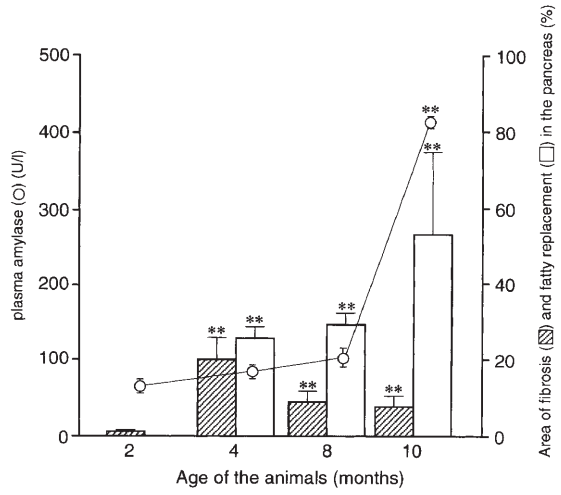

Fig. 3 Age-related changes in the areas of fibrosis and fatty replacement, and plasma levels of amylase of male WBN/Kob rats. The area of fibrosis is largest at 4 months and decreases with time thereafter. The area of fatty replacement increases with time. Plasma levels of amylase also tend to increase with time, a significant increase being seen at 10 months of age. Data are expressed as mean±SD (n=5). **P<0.01 (significant differences from 2 months of age)

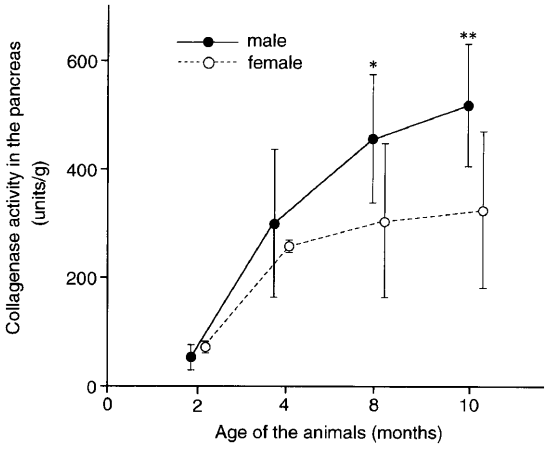

Fig. 5 Age-related changes in collagenase activity in the pancreas of male and female WBN/Kob rats. The levels are higher in males than in females as early as 4 months, and increase markedly with time. The levels in males at 8 and 10 months of age indicate significant differences from that at 2 months of age, but no significant differences were noted from levels in females. Data are expressed as mean±SD (n=5). *P<0.05, **P<0.01 (significant differences from 2 months of age)

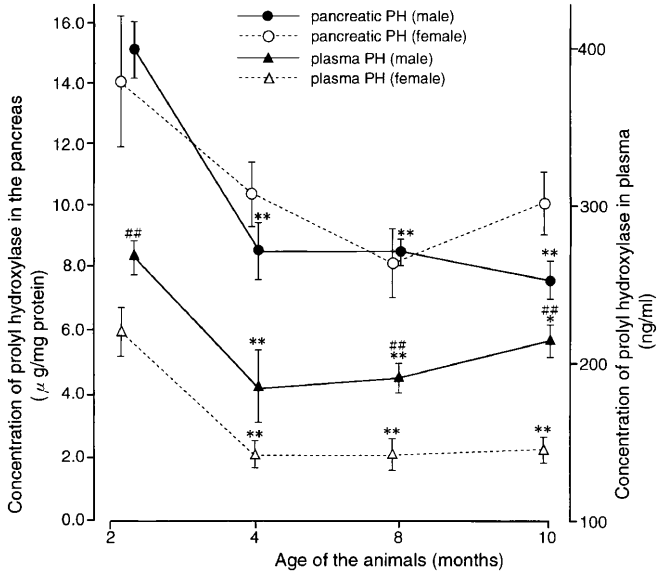

Fig. 4 Age-related changes in the concentrations of prolyl hydroxylase in the pancreas and plasma of male and female WBN/Kob rats. The plasma levels in males tend to increase with time. Data are expressed as mean±SD (n=5). **P<0.01 (significant differences from 2 months of age); ## P<0.01 (significant differences from the females)

respectively. In contrast, the areas of fatty replacement at 4 months of age were an average of 25.7±3.2%, and thereafter they increased in a time-dependent manner.

The level of amylase in the plasma from male rats at 3 months of age was 687±40 U/l. Thereafter, it increased in a time-dependent manner throughout the experimental period (Fig. 3). The concentration (269±16 ng/ml) of prolyl hydroxylase in the plasma from male rats at 2 months of age was significantly higher than that from females at the same age (230±14 ng/ml). At 4 months of age, the concentrations in males and females decreased to 185±52 and 144±19 ng/ml, respectively. Thereafter, that in males increased slightly, but the level in females showed no more conspicious changes (Fig. 4).

The concentration (15.7±2.1 μg/mg protein) of prolyl hydroxylase in the pancreata taken from male rats at 2 months of age was slightly higher than that (14.4±5.5 μg/mg protein) in females of the same age. At 4 months of age the level in males and females dropped, to 8.6±2.0 and 10.4±2.3 μg/mg protein, respectively. Thereafter, the concentration in males did not change until 8 months of age, when it started to fall, showing a further slight decrease at 10 months of age. In contrast, the enzyme level in females decreased continuously until they reached 8 months of age, and then increased when they were 10 months old (Fig. 4).

Pancreatic collagenase activity (40±43 U/g) in male rats at 2 months of age was lower than that (60±10 U/g) in females at the same age. At 4 months of age, the activity levels in males and females had increased noticeably to 279±305 and 216±89 units/g, respectively. Thereafter, the activity in males continued to increase noticeably in a time-dependent manner, but that in females increased only slightly (Fig. 5).

Discussion

In male WBN/Kob rats, fibrosis in the pancreas occurred as a continuous event following acute pancreatitis. The nodular pattern of fibrosis in the rats at 4 months of age may reflect the scattered acinar cell necrosis at 2 or 3 months of age. Klöppel et al. [9, 10] report that the focal

nature of chronic pancreatitis in humans reflects a focal process in acute pancreatitis. The features of pancreatitis and expanding manner of fibrosis in our study were in accordance with previous reports [15, 23]. We also confirmed that the fibrosis progressed in a time-dependent manner until the rats reached 4 months of age. We examined the rats at older ages and found that the fibrosis progressed temporarily and decreased later. The results of morphometric analysis supported the histopathological findings and revealed that the pancreatic fibrosis in these rats was transient.

We were able to characterize the fibrosis in male WBN/Kob rats. First of all, the fibrotic area, which progressed in the intralobular space in a mostly nodular fashion, was mainly composed of collagen. In addition, type-III collagen was demonstrated immunohistochemically in the fibrotic area, though type-I collagen was negative in the same area.

In humans, pancreatic fibrosis has been classified into interlobular and intralobular types [13, 18, 19]. Patients with chronic alcoholic pancreatitis characteristically show the interlobular type; and those with alcohol dependence syndrome, mainly the intralobular type [18, 19]. The difference between interlobular and intralobular fibrosis in humans was also demonstrated by immunohistochemistry: the former was positive for both type-I and type-III collagen, whereas the latter was positive for type-III collagen only [18]. Suda et al. [17] also report that fibrosis after acute pancreatitis may contain type-III collagen, which is ultimately followed, irreversibly, by type-I. The mechanism involved in the decrease in fibrosis in male rats may possibly have some association with collagen fibre composition, especially since Kennedy et al. [5] report that type-III collagen is relatively unstable in comparison with type-I and that deposition of type-III collagen or fibronectin, both of which appear in acute pancreatitis, is largely reversible. With all these facts taken together, it seems plausible that the pancreatic fibrosis in male rats disappears because their fibrous components are mainly reversible type-III collagen. The pancreatic fibrosis in male rats, which develops in the intralobular space, was composed mainly of type-III collagen and was not followed by deposition of type-I collagen throughout the period of study. Thus, this fibrosis was potentially reversible and the morphology in the chronic stage showed different features from that of chronic pancreatitis in humans.

The areas positive for α-smooth muscle actin also changed in accordance with the development of the fibrosis. Their transient nature may be related to healing of inflammatory injuries. In general, α-smooth muscle actin is known to be expressed in pericytes and/or smooth muscle cells, and we also recognized α-smooth muscle actin in smooth muscle cells of vessels in sites without lesions in pancreatic tissue from WBN/Kob rats. However, in granulation tissues, α-smooth muscle actin was reported to be transiently expressed and to abate when the wounds had healed; expression was considered to be brought about by a differentiation of myofibroblasts from granulation fibroblasts [1]. We believe that the transient expression of α-smooth muscle actin in the fibrotic area in the pancreas from male rats may occur by the same mechanisms as operate in wound healing.

Based on the analytical results of the plasma amylase levels, it is clear that destruction of acinar cells continued after the pancreatitis entered the chronic stage [22]. This may indicate that relapsing pancreatitis occurred in male WBN/Kob rats.

Prolyl hydroxylase is an enzyme involved in the synthesis of collagen [16, 24] and plays an important part in pancreatic fibrosis [21]. Our results show that the plasma levels of prolyl hydroxylase in male rats were higher than those in females throughout the experimental period. Thus, these findings seem to reflect the collagen-synthesizing activity in the pancreas of male rats. However, in terms of the levels of the prolyl hydroxylase in the pancreas no sex difference was evident. We attribute this discrepancy to the method used for analysis of prolyl hydroxylase: the levels of prolyl hydroxylase were determined per milligram of protein in our study and would thus be affected by protein that enters the pancreatic tissue in association with the inflammation. From another point of view, these results suggest that plasma rather than pancreatic levels of prolyl hydroxylase would be more useful for the diagnosis of chronic pancreatitis or pancreatic fibrosis in humans. In male and female WBN/Kob rats, the levels of prolyl hydroxylase in both their plasma and their pancreas were higher at 2 months of age than later. We consider that this finding reflects the maturation of the pancreas, since the pancreatic connective tissue is abundant in the prenatal and newborn periods and decreases in infancy [3].

The results of the determination of collagenase activity in the pancreas suggested a possible mechanism for the decrease in pancreatic fibrosis. Collagenase activity in pancreata from male rats at 2 months of age was slightly lower than that in pancreata from females at the same age. Thereafter, the activity in the former increased in a time-dependent manner. In contrast, that in the latter increased until 4 months of age and showed no remarkable changes later. Pancreatic collagenase is known to increase in pathologic conditions, including necrotizing pancreatitis in rats [8]. However, there is a report indicating that collagenolytic cathepsin increased in ethanol-fed rats without an increase in the collagenase activity [24]. We propose, then, that at least collagenase acts in the degradation of collagen fibres to reduce the fibrosis in male rats, even though prolyl hydroxylase is maintained at a high level and collagen synthesis continues.

In conclusion, male WBN/Kob rats developed acute pancreatitis at 2–3 months of age, and the lesions later underwent widespread fibrosis in the intralobular space. Thereafter, the fibrosis decreased and adipose tissue replaced the fibrous tissue. The fibrotic area in these rats was mainly composed of type-III collagen. The transitional change in fibrosis may be a result of development of type-III collagen as a fibrotic component and the balance between the prolyl hydroxylase level and collagenase activity.

References

1. Darby I, Skalli O, Gabbiani G (1990) α-Smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest 63:21–29
2. Ectors N, Maillet B, Aerts R, Geboes K, Donner A, Borchard F, Lankisch P, Stolte M, Lüttges J, Kremer B, Klöppel G (1997) Non-alcoholic duct destructive chronic pancreatitis. Gut 41:263–268
3. Hoshino A, Suda K, Hashimoto K (1980) Development of pancreas in childhood. Juntendo Med J 26:63–68
4. Ishida K, Mizuno A, Sano T, Shi K, 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 129:585–593
5. Kennedy RH, Uscanga L, Choux R, Grimaud J-A (1984) Pancreatic connective matrix changes in acute pancreatitis. In: Gyr KE, Singer MV, Sarles H (eds) Pancreatitis: concepts and classification. Elsevier Science, Amsterdam, pp 147–148
6. Kishino Y, Kawamura S (1984) Pancreatic damage induced by injecting a large dose of arginine. Virshows Arch [B] 47:147–155
7. Kissane JM, Lacy PE (1990) Pancreas and diabetes mellitus. In: Kissane JM (ed) Anderson's Pathology, 9th edn. Mosby, St. Louis, pp 1347–1346
8. Kinami Y, Takata M, Miyazaki I (1983) An experimental study on pancreatic fibrosis and collagenase activity following acute pancreatitis. Jpn J Gastroenterol 80:91–97
9. Klöppel G, Maillet B (1992) The morphological basis for the evolution of acute pancreatitis into chronic pancreatitis. Virchows Arch [A] 420:1–4
10. Klöppel G, Maillet B (1993) Pathology of acute and chronic pancreatitis. Pancreas 8:659–670
11. Kodama S, Uchijima E, Nagai M, Mikawatani K, Hayashi T, Suzuki K (1990) One-step sandwich enzyme immunoassay for soluble human thrombomodulin using monoclonal antibodies. Clin Chim Acta 192:191–200
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
13. Martin ED (1984) Different pathomorphological aspects of pancreatic fibrosis, correlated with etiology: anatomical study of 300 cases. In: Gyr KE, Singer MV, Sarles H (eds) Pancreatitis: concepts and classification. Elsevier Science, Amsterdam, pp 77–83
14. Miyawaki S, Nakamura Y, Suzuka H (1994) A new mutation, aly, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. Eur J Immunol 24:429
15. Ohashi K, Kim JH, Hara H, Aso R, Akimoto T, Narama K (1990) WBN/Kob rats, a new spontaneously occurring model of chronic pancreatitis. Int J Pancreatol 6:231–247
16. Richard AB, Darwin JP (1973) Affinity column purification of protocollagen proline hydroxylase from chick embryos and further characterization of the enzyme. Biol Chem 248:1175–1182
17. Suda K, Tsukahara M (1992) Histopathological and immunohistochemical studies on apparently uninvolved area of pancreas in patients with acute pancreatitis. Arch Pathol Lab Med 116:934–937
18. Suda K, Akai J, Nakamura T (1993) Pancreatic fibrosis in patients with alcohol dependence syndrome. Arch Pathol Lab Med 117:1013–1016
19. Suda K, Shiotsu H, Nakamura T, Akai J, Nakamura T (1994) Pancreatic fibrosis in patients with chronic alcohol abuse: correlation with alcoholic pancreatitis. Am J Gastroenterol 89:2060–2062
20. Suda K, Takase M, Takei K, Nakamura T, Akai J, Nakamura T (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
21. Suda K, Hosokawa Y, Abe H, Kuroda J, Suzuki F, Kamano T (1998) Immunolocalization of the β subunit of prolyl hydroxylase in the pancreas. Pancreas 17:98–100
22. Takahashi M, Nagase S, Takahashi M, Kokubo T, Hayashi Y (1981) Changes of amylase during experimental pancreatic carcinogenesis in hamsters. Gann 72:615–619
23. Tsuchitani M, Saegusa T, Narama I, Nishikawa T, Gonda T (1985) A new diabetic strain of rat (WBN/Kob). Lab Anim 19:200–207
24. Yamada M, Murawaki Y, Hirayama C (1987) Effects of ethanol feeding on collagen synthesizing and degrading enzymes in rat pancreas. Biochem Pharmacol 36:3361–3363