◎総　説

Experimental model of chronic pancreatitis, a review —Does it really exist ?

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Abstract : Experimental model of pancreatitis is mandatory for elucidating the pathobiology of the disease and also to see the response of a novel treatment. In addition, the need for an animal model of chronic pancreatitis is further strengthened by the relative inaccessibility and paucity of the human pancreatitis tissue. Whereas various models of acute pancreatitis and also of exocrine pancreatic tumor have been described, chronic pancr-eatitis has not been consistently reproduced in experimental animals. Many researchers attempted to establish an experimental model of chronic pancreatitis either by partially obstructing the drainage of pancreatic secretion in dogs and cats or by feeding alcohol to dogs and rats with and without temporary occlusion of the biliopancreatic duct or by surgically inducing ischaemia in the pancreas of the dogs. But, none of these models is identical with human disease. A consistently reproducible model of human chronic pancr-eatitis probably does not exist. In this expanding era of molecular biology which promises us to enhance greatly our understanding of this disease, a right experimental model of chronic pancreatitis is still in progress.

Introduction

Human chronic pancreatitis is characterized morphologically by an irregular sclerosis with destruction and permanent loss of exocrine and endocrine parenchyma which may be either focal, segmental or diffuse. This may be associated with variable degrees of dilation of segments of the duct system (1). Worldwide, the most common forms of chronic pancreatitis are related to alcohol ingestion, the poorly understood entity of

"tropical pancreatitis" and an idiopathic variant. Other causes of pancreatitis such as hypercalcemia, hyperparathyroidism, trauma, hereditary factors are rare. (2). All concepts of the pathophysiology of chronic pancreatitis remain controversial, at least in part (2). The relative unavailability of the pancreas tissue leads the investigator to diagnose and grade the disease on the basis of imaging techniques. To correlate the etiological factors with the definite pathogenetic mechanism, it is mandatory to create an experimental model of chronic pancreatitis.

An experimental model of chronic pancreatitis probbly does not exist. Whereas various models of acute pancreatitis and exocrine pancreatic tumor have been described (3, 4), chronic pancreatitis has not been consistently produced in experimental animals.

An ideal experimental model of any disease should resemble the clinical disease in all features such as etiology, pathophysiology, pattern of progression, response to therapy and sequeale. Such an ideal model probably does not exist, except for mostly infectious diseases (22).

Many researchers attempted to create an experimental models of chronic pancreatitis but none of these models are really comparable either morphologically or functionally to human chronic pancreatitis. To date, at least six animal models of chronic pancreatitis have been described :

1. Experimental pancreatolithiasis in dogs (5, 6, 7, 8, 9)
2. Alcoholic pancreatitis in dogs and rats fed ethanol for 2 years (10, 11, 12, 13, 14, 15).
3. Alcohol induced chronic pancreatitis in rats after temporary occlusion of biliopancreatic ducts with Ethibloc (16, 17).
4. Feeding of rats with a protein deficient diet (20, 21).
5. Chronic pancreatitis in cats (19).
6. Canine model of chronic pancreatitis due to chronic ischaemia (18).

Experimental Pancreatolithiasis in the Dog

Floyd and Christopherson (5) first attempted to create a model of chronic pancreatitis by ligating the main pancreatic duct. These authors failed to produce chronic pancreatitis by simple injection of a fibroblastic agent sodium dicetyl phosphate into the wall of the major pancreatic duct of the dog (5), but they observed chronic interstitial pancreatitis with ductal dilation in dogs by injecting the wall of the major pancreatic duct with the sodium dicetyl phosphate and placing a polythylene collar about the site of injection. The pathologic process produced by this method appears sufficiently comparable to that seen in man.

Since total ligation of the pancreatic duct produced no chronic pancreatitis, Japanese scientists have tried to develop a model of chronic pancreatitis and pancreatolithiasis by partial ligation of the major pancreatic duct in dogs. Konishi et al (6) could accomplish an experimental model of pancreatolithiasis in mongrel dogs by the partial obstruction of the pancreatic excretion by partial ligation of the major pancreatic duct. They found that pancreatic calculi were demonstrated in 46.7% of the dogs with partial outflow obstruction at 4 months, whereas no pancreatic calculi were found in any of the dogs with complete duct obstruction. All calculi produced were localized in the ductal system and the organic and inorganic composition of

canine and human pancreatic calculi were quite similar. Connective tissue proliferation and mucous cell metaplasia of the ductal epithelium, often seen in association with clinical pancreatolithiasis, were also observed in the pancreata of the dogs that have had partial obstruction of the pancreatic excretion.

Ultrastructural changes in the exocrine pancreas in this canine model of pancreatolithiasis were carried out by Sakakibara et al (7). Light microscopy demonstrated pancreatic lesions similar to those in humans. Electron microscopy revealed dilated lumens of small ducts and degenerated ductal cells 3 months after the ligation. These changes became more severe and appeared more frequently when the period of ligation was prolonged up to 1 year. Acinar cells demonstrated dilatation of the rough endoplasmic reticulum and the golgi apparathsu, swelling of the mitochondria and an increase in the number of the prozymogen granules. Microfilamentous substances appeared in markedly dilated rough endoplasmic reticulum and in the intercellular space as acinar cell lesions progressed and the basal membrane became disrupted. The substances might be involved in calculous formation, the incidence of which reached a plateau after six months of ligation, coinciding with the peak of the substance.

In dogs with partial ligation of the major pancreatic duct advanced insufficiency of the exocrine pancreas, evaluated by caerulein/secretin test, was observed after six months (8).

Endocrine function was also assessed by Okamura et al (9) in this experimental pancreatolithiasis model in dogs. Endocrine insufficiency of the pancreas observed in this experimental model was similar to that reported in human pancreatolithiasis, although the severity was less in dog. Endocrine functoin was serially examined by an intravenous glucose (0.5 g/kg) tolerance test and an insulin (0.5U/kg) tolerance test before and 3, 6, 12 months after the duct ligation. Neither alpha nor beta cell dysfunction became apparent untill 12 months after the ligation. The disappearance rate of glucose in an intravenous glucose tolerance test decreased from 2.92 ± 0.41 (mean ± SE) of the pre-ligation value to 1.58 ± 0.17%/min at the end of the 12 months period ($p<0.02$). Plasma insulin response to glucose was also reduced significantly. Although hypoglycaemia induced during an intravenous insulin tolerance test was maximal in the 12th month, distinct response of plasma pancreatic glucagon to the hypoglycaemia disappeared.

Austin et al (19) have described a canine model of obstructive chronic pancreatitis in cats by narrowing the main pancreatic duct to about 25% of its original diameter preserving the drainage of the accessory lobe and the head of the gland. The author described the histological and functional picture similar to that of clinical chronic pancreatitis (19).

Chronic Alcoholic Pancreatitis in Rats and Dogs

Sarles et al (10) described alcoholic pancreatitis in rats which resembles that of human chronic alcoholic pancreatitis. In their study, 45 Wistar rats fed on a balanced diet, were given 20% ethanol freely for 20 to 30 months. More than half the animals developed pancreatic lesions very similar to those of human disease. The pathological changes

in foci surrounded by normal pancreatic tissue, were a reduction in acini, duct multiplication (probably by neogenesis), protein plugs, sometimes calcified in the ducts and fibrosis. Samples of pancreatic juice from four animals exposed to ethanol contained significantly higher protein concentrations than those samples taken from two control animals. Protein precipitates appeared spontaneously in the pancreatic juice of the animals exposed to ethanol, but not in that of the controls. Beta cell adenomata of the islets of Langerhans were observed in four of the rats exposed to ethanol.

Sarles et al have described chronic alcoholic pancreatitis also in Dogs (11, 12). The pancreas in dogs receiving alcohol at 2 g／kg per day for 1 − 3 years was frequently normal. In some cases, however it was possible to find intraductal plugs, periductal fibrosis, duct proliferation and less frequently perilobular and intralobular fibrosis (11).

Changes in pancreatic exocrine function were observed in these alcoholic dogs which also received a diet rich in fat and protein. From the 16th week they began to excrete protein precipitates through the pancreatic cannula. After two years of alcohol adminstration, pancreatic biopsies showed lesions which were comparable with the early stages of human calcifying chronic pancreatitis and obstruction of the ducts by protein precipitates, some of which were calcified, atrophy or hyperplasia and duplication of ducts, pericanalicular fibrosis, and atrophy of acinar cells (11, 12).

But other investigators using similar ethanol feeding protocols were unable to consistently to produce alcoholic pancreatitis in rats (13, 14).

Singh et al tried to develop a model of chronic alcoholic pancreatitis in Sprague-Dawley rats fed nutritionally adequate diets (15). Three groups of 15 animals each were fed Wayne Rodent-Blox ad libitum, Lieber-DeCarli diet with 40% of carbohydratye calories replaced by ethanol ad libitum and isocaloric amounts of Lieber-De Carli diet respectively for a period of 18 months. All of the ethanol-fed animals developed morphological changes akin to human chronic pancreatitis. There were focal areas of parenchymal degeneration with fibrosis, protein plug formation and tubular complexes. Based on biochemical data Singh (15) suggested that focal degenerative changes may be due to trypsin generated by intracellular activation of digestives enzymes by lysosomal enzyme cathepsin B.

Alcohol-induced Chronic Pancreatitis in Rats After Temporary Occlusion of Biliopancreatic Ducts with Ethibloc

Pap and Boros (16) and Pap et al (17) provoked chronic obstructive pancreatitis like histological and biochemical alterations in male wistar rats with ethibloc occlusion of the common bile ducts and the main pancreatic ducts. After the disappearance of the glue from the ducts, a gradual and almost total recovery was demonstrated during a 2 month observation period. About 12 g／kg alcohol (20% vol／vol) given daily by gastric intubation and ad libitum intake inhibited the recovery of pancreatic weight and enzyme contents in the occluded rats, and within a 2 month period chronic calcifying type pancreatitis became evident with some signs of remaining obstructive pancreatitis like

lesions. Cessation of alcohol adminst-ration after 2 months resulted in a recovery of pancreatic weight and enzyme contents, although morphological regeneration was less pronunced and calcification remained visible in some rats. A 50% raw soy flour diet provoked some further changes in the proportion of intra-pancreatic enzymes without any supplementary increase of pancreatic weight and protein content. The authors concluded that chronic obstructive and chronic calcifying pancreatitis can appear together and earlier if the etiological factors act in combination. Suppression of pancreatic regeneration by alcohol seems to be necessary to maintain chronic pancreatitis-like lesions and to develop calcification (16).

Canine Model of Chronic Pancreatitis due to Chronic Ischaemia

Tanaka et al (18) described an experimental model of chronic pancreatitis which was produced by a chronic ischaemia which was induced by ligation and separation of branches flowing into the left pancreatic lobe from the splenic artery. Macroscopic finding at 3 and 6 months after model preparation showed that the pancreas was hard, with severe inflammatory changes. In the secretin-cerulein test at 3 and 6 months, the flow rate of pancreatic juice, amylase output and bicarbonate concentration were significantly reduced as compared with the controls. The histopathological findings consisted of a decrease in the pancreatic parenchyma, replacement with fat, severe inflammatory cell infiltration, extensive fibrosis and tubular complexes. As this model closely resembled human chronic pancreatitis, the authors concluded (18) that ischaemia might be an etiological factor in human chronic pancreatitis.

Models for Tropical Pancreatitis

Tropical calcific pancreatitis is a nonalcoholic type of chronic pancreatitis affecting the children and young adults characterized clinically by recurrent abdominal pain in childhood, diabetes in adolescent and death in early adult life (20). Although the exact etiology is not known, malnutrition and chronic cassava toxicity, either singly or in combination are presumed to be the prime factor in pancreatic injury unopposed by detoxification of free radicals (20).

Clinical and experimental studies have substantiated that the pancreas is quite vulnerable to protein deprivation. A generalized reduction in the size of the pancreas, atrophy, fibrosis, disorganization, and loss of acinar pattern are characteristic of pancreatic injury seen in kwashiorkor. Pancreatic function is markedly depressed in protein malnutrition. Experimental studies designed to study the effect of protein malnutriton on animals have confirmed the clinical observations. After 10 days of protein free diet, the acinar cell of the rat pancreas showed coarsening of nuclear matrices, depletion of zymogen granules, loss of chromosomal material and separation of the tubules of the endoplasmic rerticulum (21).

Despite the availability of good experimental models of pancreatic insufficiency secondary to protein deficiency reproducing kwashiorkor, no existing model reproduces the lesions of human chronic pancreatitis, with its progressive development and high incidence of calcium protein precipitates in the ducts.

Conclusion

Despite the fact that partial obstruction of the drainage of the pancreatic secretion in dogs and cats, feeding alcohol to dogs and rats with and without temporary occlusion of the biliopancreatic duct, and surgically inducing ischaemia in the pancreas in dogs have been found to produce functional and morphological changes in the pancreas, none of these models is identical with the human disease.

The term 'chronic' has to be questioned since it varies from periods of 2 weeks to 2 years in study to study. These time intervals are much shorter than those seen in humans in whom it takes usually more than 10 years of chronic alcoholism to develop chronic calcifying pancreatitis.

The relative inaccessibility of human chronic pancreatic tissue really poses a barrier in elucidating the pathophysiology of this disease and in vitro studies alone probably will not help to solve the riddle of human chronic pancreatitis. On the other hand molecular biology is expanding day by day, which promises us to enhance greatly our understanding of this disease. So the search for the right animal model of chronic pancreatitis is stiil in progress.

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"慢性膵炎の実験モデル"

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疾患の実験モデルの作成は，その疾患の病因，病態の解明，さらに治療法の開発のために重要である。筆者らの一人は厚生省難治性膵疾患調査研究班の班員として，慢性膵炎の病態の解明や治療法の開発に関する研究を行っており，その研究の一環として，慢性膵炎の実験モデルの作成を現在行っている。そこで，これまで報告されている慢性膵炎の実験モデルについて概要を報告した。種々の動物や方法でヒト慢性膵炎に病因，病態，組織像が類似するモデルの作成が試みられてきたが，そのすべてが合致するような慢性膵炎モデルは確立されてはいない。近年の分子生物学的研究の進歩は著しく，実験モデルへの応用が種々なされている現在，より簡便で再現性のある慢性膵炎モデルの作成が望まれるところである。