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Experimental Models of Pancreatitis

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Acute pancreatitis is an inflammatory disease characterized by interstitial edema, inflammatory cell infiltration, and acinar cell necrosis, depending on its severity. Regardless of the extent of tissue injury, acute pancreatitis is a completely reversible process with evident normal tissue architecture after recovery. Its pathogenic mechanism has been known to be closely related to intracellular digestive enzyme activation. In contrast to acute pancreatitis, chronic pancreatitis is characterized by irreversible tissue damage such as acinar cell atrophy and pancreatic fibrosis that results in exocrine and endocrine insufficiency. Recently, many studies of chronic pancreatitis have been prompted by the discovery of the pancreatic stellate cell, which has been identified and distinguished as the key effector cell of pancreatic fibrosis. However, investigations into the pathogenesis and treatment of pancreatitis face many obstacles because of its anatomical location and disparate clinical course. Due to these difficulties, most of our knowledge on pancreatitis is based on research conducted using experimental models of pancreatitis. In this review, several experimental models of pancreatitis will be discussed in terms of technique, advantages, and limitations.

Key Words: Pancreatitis; Models; Animal; Experiment

INTRODUCTION

Acute pancreatitis and chronic pancreatitis are inflammatory disorders with diverse etiologies. About 85% to 90% of acute pancreatitis patients show a rather benign clinical course that is mostly self-limited and responds well to conservative treatment. Meanwhile, necrotizing pancreatitis, which accounts for the remaining 10% to 15% of cases, follows a grave clinical course that is more often accompanied by local/systemic complications and organ failure. Despite the considerable progress that has been made over the past decades, the treatment of acute pancreatitis remains supportive, and for the time being there are no specific treatments that can alter the course of the disease. This lack of target therapy is mainly due to our incomplete understanding of the underlying mechanism of acute pancreatitis.[1]

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Although many etiologies have been proposed in the pathogenesis of acute pancreatitis, current knowledge on how each induces the inflammatory process, yields diverse clinical presentations, and at times results in organ failure is far from satisfactory. Thus, a better understanding of the pathogenesis of pancreatitis is needed, and this will enable us to develop more effective treatment modalities and strategies that will ultimately prevent or decrease the incidence of diverse pancreatitis-related complications. However, because of the anatomical location of the pancreas and the difficulty in procuring tissue at different stages of the inflammatory process in humans, our understanding of the pathogenesis of pancreatitis mainly relies on data from experimental animal models.[2]

Chronic pancreatitis is an irreversible inflammatory process with morphological changes that are mainly characterized by interstitial fibrosis and acinar cell atrophy. Although alcohol consumption is one of the major etiologies of chronic pancreatitis, a clinically relevant animal model has yet to be established. The identification and characterization of the stellate cell has led to the development of several animal models aimed at clarifying the mechanism of acinar cell injury and subsequent interstitial fibrosis.[3] This article is intended as an introduction to representative experimental animal models of acute pancreatitis and also as a survey of some promising experimental animal models of chronic pancreatitis currently un-

der extensive study.

ANATOMY OF THE PANCREAS IN EXPERIMENTAL ANIMALS

Some differences exist between human pancreases and pancreases of experimental animals used for research purposes. Understanding these differences is therefore critical to carrying out experiments and analyzing their results. The main difference between the human pancreas and the rat pancreas is that in humans, the bile and pancreatic ducts are separate ducts that normally form a very short common channel in the duodenal wall, whereas those of the rat form a long common channel with the bile duct also serving as a conduit of pancreatic juice.[4] Another noticeable difference is the absence of a gallbladder in rats. In addition, the rat pancreas is composed of multiple segments in the shape of an omentum, while the human pancreas is a single solid organ abutting the duodenal wall. Despite these differences, the rat pancreas also shares many similarities with the human pancreas. First, the pancreatic duct drains into the duodenum. Second, there is a similarity of cellular components, including acinar, ductal, stellate, and endocrine cells. Third, the rat pancreas also serves to maintain both exocrine and endocrine functions.

EXPERIMENTAL MODELS OF ACUTE PANCREATITIS

Cerulein-induced acute pancreatitis model

Pancreatitis induced by cerulein is the most widely used experimental animal model of acute pancreatitis. This model is highly reproducible and economical since it utilizes rats or mice. The model has been used extensively in research settings, and the pathogenesis of acute pancreatitis induced by this agent is therefore quite well understood.[5,6]

Acute pancreatitis can be induced by an intravenous or intraperitoneal injection of an overdose of cerulean, that is, 5 μg/kg/hr in rats and 50 μg/kg several times at hourly intervals in mice. According to previous studies, cerulein is known to induce pancreatic enzyme activation within 30 minutes of intravenous administration. Many findings comparable to those of acute pancreatitis in humans, such as the following, are reproduced in this cerulein-induced acute pancreatitis model, including hyperamylasemia and diverse histopathological findings: infiltration of inflammatory cells within the pancreas, pancreatic edema, acinar cell vacuolization, and the presence of activated pancreatic enzyme within the pancreas.[6] Apart from injury to the acinar cells, ductal and endocrine cells are not damaged. Moreover, cerulein-induced pancreatitis completely resolves after cerulein is withdrawn. Since the histopathological findings in cerulein-induced acute pancreatitis closely resemble those of acute pancreatitis in humans, this model is considered as a representative model of mild acute pancreatitis and is widely used to study the pathogenesis of acute pancreatitis in terms of intracellular enzymatic activation and mechanisms of inflammatory cell infiltration. Nevertheless, this model has been criticized for its inability to accurately portray the clinical situation, which includes hypersecretion of cholecystokinin (CCK). However, considering the fact that pancreatic duct obstruction or alcohol consumption does not always induce pancreatitis and that acute pancreatitis cannot always be predicted, devising a model that perfectly reflects the clinical scenario is not possible. Therefore, the aforementioned numerous similarities between cerulein-induced acute pancreatitis and human acute pancreatitis sufficiently justify the use of this model for the study of acute pancreatitis.

Study results on the underlying mechanism by which cerulein overdose induces acute pancreatitis can be summarized as follows.[6,7] Cerulein is a hormone that is analogous to CCK, which induces pancreatic enzyme secretion. Two CCK receptors, namely high and low affinity CCK receptors, have been identified. As the name suggests, the high affinity CCK receptor shows a high affinity for CCK and couples with CCK to induce exocytosis of zymogen granules from the acinar cells, thereby increasing the secretion of digestive enzymes. Meanwhile, the low affinity CCK receptor has a low affinity for CCK and couples with CCK after the high affinity CCK receptors have been saturated. When CCK binds to the low affinity CCK receptor, it inhibits the secretion of digestive enzymes from acinar cells. Thus, the response of the CCK receptor to CCK is biphasic; that is, stimulation is followed by inhibition. Therefore, when pancreatic acinar cells are stimulated by an excessive amount of CCK, exocytosis of zymogen granules from the acinar cells is inhibited, and this leads to the accumulation of digestive enzymes within the acinar cells. It is well known that digestive enzymes in the zymogen granules are stored as proenzymes in inactive forms. However, the method by which these enzymes become activated within the pancreas and induce pancreatitis remains unclear. Unraveling this process is critical to identifying the underlying pathogenesis of pancreatitis. In the cerulein-induced acute pancreatitis model, the histological changes of the pancreas are mild, progress within a few hours, and completely resolve after a period. Therefore, this model is suitable for investigating several aspects of acute pancreatitis, such as cellular changes observed in the early phases of acute pancreatitis and the autoactivation process of digestive enzymes. Since acute pancreatitis can be induced by cerulein in many different animals, including mice and rats, it has the potential of being widely applied in a variety of settings. This model is especially useful and most appropriate for

investigating the pathogenesis of acute pancreatitis with genetically engineered mice.

Bile salt-induced acute pancreatitis model

The bile salt-induced acute pancreatitis model was first reported by Aho et al.[8,9] and has been widely accepted as a representative model of severe acute pancreatitis that shows pancreatic necrosis. This type of acute pancreatitis is induced by first inserting the cannula through the orifice that opens into the duodenum and infusing 0.2 to 0.3 mL of 3% to 5% sodium taurocholate in a retrograde fashion. Since the pancreatic and bile ducts form a long common channel, it is necessary to temporarily block the bile duct at the level of the hilum with forceps to prevent the reflux of sodium taurocholate into the liver. In addition, the cannula should be removed after infusing the predetermined amount of sodium taurocholate to allow and maintain a normal flow of bile and pancreatic juice through the duct. Disadvantage of this model is the requisite abdominal incision that has to be made to carry out the experiment and the expertise required to insert the cannula through the pancreatic duct orifice. Although the mechanism by which sodium taurocholate infusion induces acute pancreatitis is yet unclear, it is postulated to be attributable to the detergent effect of the bile salt. Immediately after sodium taurocholate infusion, hemorrhagic necrosis can be observed in the pancreatic parenchyma surrounding the main pancreatic duct. When the suture site is opened again 6 to 12 hours after the abdominal wall is closed, ascites and severe hemorrhagic necrosis of the pancreas can be observed. The advantage of this model is that severe hemorrhagic necrosis of the pancreas can be induced in a relatively short period and that consequent multiorgan failure can be investigated. However, since the damage to the pancreas is not uniform and is mainly limited to the vicinity of the main pancreatic duct, sampling error is likely to occur when the histological changes of the pancreas are studied or experiments using tissue samples are carried out.

Choline-deficient, ethionine-supplemented diet pancreatitis model

When 4- to 6-week-old female mice weighing 10 to 14 g are allowed to feed freely on a choline-deficient, ethionine-supplemented (CDE) diet, acute hemorrhagic pancreatitis is induced within about 5 days with a mortality rate reaching nearly 100%.[10] This was done by placing the mice on nil per os except for water for the first 24 hours, then allowing 3 g of CDE diet per mouse for the next 24 hours and checking the mortality rate every 24 hours thereafter. The disadvantage of this experimental model is that the occurrence of acute pancreatitis is somewhat limited to young female mice. However, a relatively constant mortality rate is highly advantageous for evaluating the efficacy of new drugs being developed to treat pancreatitis.

L-arginine-induced acute pancreatitis model

L-arginine is an essential amino acid that has been used to induce severe necrotizing acute pancreatitis in rats. Tani et al.[11] demonstrated that a single intraperitoneal injection of an excessive dose of L-arginine (500 mg/100 g body weight) induces acute necrotizing pancreatitis in rats. Histological examination revealed degenerative changes of intracellular organelles and nuclei of acinar cells. The extent and severity of necrotic changes in pancreatic exocrine tissue with inflammatory cell infiltration were maximal at 72 hours. Pancreatic acinar cells began to regenerate within 7 days, and pancreatic architecture appeared almost normal after 14 days. However, the pathogenic mechanism is not yet fully understood.

Pancreatic duct ligation model

The changes observed in the pancreas after ligation of the pancreatic duct vary according to the animals used. One hypothesis on the pathogenesis of pancreatitis in humans is the common channel hypothesis proposed by Opie et al.[12] This hypothesis suggests that when the ampullary orifice is obstructed by a gallstone, bile refluxes into the pancreatic duct and induces acute pancreatitis. However, since pancreatic duct pressure is greater than that of the bile duct, it is known that pancreatic juice refluxes into the bile duct in the presence of ampullary orifice obstruction and not vice versa. Furthermore, pancreatitis rarely occurs in patients with cancer in the ampulla of Vater, even after the pancreatic duct has been obstructed by the tumor. Therefore, the validity of this hypothesis is questionable.

When the pancreatic duct is ligated in rats, initial findings such as pancreatic edema, inflammatory cell infiltration, hyperamylasemia, and so on are compatible with acute pancreatitis.[13] With time, these findings are replaced by findings consistent with chronic pancreatitis such as atrophy, loss of acinar cells, and fibrosis.[13,14] These changes will be discussed in greater detail later. The pancreatic anatomy of the American opossum is similar to that of humans, and when the common channel of the pancreatic duct and the bile duct is ligated, acute necrotizing pancreatitis can be induced.[15] When ligating the pancreatic duct at the common channel level, the cystic duct must be simultaneously ligated. However, apart from the disadvantage of having to perform laparotomy, purchasing and carrying out experiments with American opossums is a difficult and limited practice because domestic laboratory animal distributors do not import this species. Nevertheless, this model is clinically useful in evaluating the efficacy of drugs for pancreatitis and investigating mechanisms of multiorgan failure since the clinical scenario is consistent with that in humans by

virtue of its analogous pancreaticobiliary ductal anatomy.

EXPERIMENTAL MODELS OF CHRONIC PANCREATITIS

The pathological features of chronic pancreatitis include loss of exocrine and endocrine cell mass, infiltration of chronic inflammatory cells, formation of intraductal protein plugs, calcification, and interstitial fibrosis. Several animal models of chronic pancreatitis have been developed, each with its own merits and demerits. Despite the substantial effort that has been put into clarifying the pathogenesis and natural course of chronic pancreatitis, a clinically relevant and satisfactory animal model has not yet been established. However, the identification and characterization of the pancreatic stellate cell has instigated the development of animal models that show promising results and are expected to provide us with a better understanding of the molecular and etiological backgrounds of chronic pancreatitis.[16]

Among the various experimental models of chronic pancreatitis, the most commonly used models are surgical ligation of the pancreatic duct (obstructive), ethanol feeding (alcohol-induced), repetitive cerulein injection (recurrent pancreatitis), and toxic chemicals-induced models.

Duct obstructive model

Pancreatic duct ligation has been used for the induction of pancreatic fibrosis. However, clinical and pathological features after duct ligation are species-dependent. Duct obstruction alone in rats results in acinar cell atrophy and fibrosis without a profound inflammatory reaction. In contrast to the rat, the mouse pancreas consists of three lobes, a gastric, splenic, and duodenal lobe, which drain their pancreatic secretions into individual ducts.[4] This makes duct ligation in the mouse for the uniform induction of fibrosis technically difficult by comparison with the rat. Besides the complete obstruction of the pancreatic duct, the duct hypertension model, which was reported by Yamamoto et al.,[17] suggests that pancreatic duct hypertension (PDH) plays an important role in the initiation and development of chronic pancreatitis. However, the preparation of this model is technically challenging. Briefly, the common bile duct is ligated proximal to the pancreas near the liver, and a cannula is inserted above the ligature to collect pure bile. Another cannula is inserted into the biliopancreatic duct through the ampulla of Vater to collect pure pancreatic juice. An additional cannula is inserted into the duodenum to return bilopancreatic juice. PDH can be induced by vertically raising the free end of the pancreatic duct cannula to exert a hydrostatic pressure and maintaining it for 2 weeks. PDH is then gradually increased, but when the pancreatic juice flow becomes interrupted, PDH is decreased to restore pancreatic juice flow. The induction of PDH results in a marked reduction of amylase activity in the pancreatic juice and an increase in serum amylase activity. At 2 weeks after persistent PDH, pancreatic exocrine function shows a markedly decreased response to a bolus injection of secretin (100 pmol/kg) compared with the control. Histological examination reveals interlobular and intralobular fibroses in the form of nodular pancreatitis at 2 weeks after the induction of PDH. Immunohistochemistry shows the expression of fibronectin and collagen types I and III. Quantitative real-time reverse transcription polymerase chain reaction shows an increase in transforming growth factor- β1 mRNA expression in the pancreas during PDH.[17]

Repeated cerulein-induced chronic pancreatitis model

Recurrent episodes of acute pancreatitis lead to chronic pancreatic injury in humans. Mimicking the human pathogenesis, repeated bouts of cerulein-induced acute pancreatitis in the course of several weeks causes chronic injury to the pancreas with resultant collagen deposits and fibrosis.[17] Briefly, acute reversible pancreatic injury is induced in mice by twice weekly cerulein treatment at 50 μg/kg/hr×6 hour for 10 weeks. In this model, procollagen α1 mRNA markedly increases by week 2. Sirius red staining of interstitial collagen demonstrates the progressive accumulation of extracellular matrix surrounding the acinar units and in the interlobular spaces. Atrophy, the transdifferentiation of acinar units to duct-like tubular complexes, and dilatation of the intra-acinar lumen also developed.[18] This repeated cerulein injection animal model is widely used because it is easy to induce and shows good reproducibility.

Alcohol-induced chronic pancreatitis model

Alcohol is one of the major etiologic factors of chronic pancreatitis. Several trials have been conducted with the aim of developing a chronic pancreatitis experimental model with chronic alcohol administration. Disappointingly, however, alcohol ingestion alone did not induce chronic pancreatitis despite long experimental durations. In contrast to the liver, in which ethanol induces severe organ damage, prolonged alcohol ingestion only caused mild pancreatic injury rather than chronic pancreatitis. Nevertheless, the combination of alcohol and various agents such as cerulein or lipopolysaccharide exacerbated pancreatitis, ultimately resulting in fibrosis and a reduction of acinar cell mass.[18] In Sprague-Dawley rats fed isocaloric Lieber-DeCarli liquid diets with alcohol for 10 weeks and challenged with a single dose or three repeated doses of the endotoxin lipopolysaccharide, stellate cell activation and fibrosis occurred.[19]

Toxic chemical-induced pancreatitis

Dibutyin dichloride (DBTC) is a chemical that is industrially used for the heat stabilization of polyvinylchloride plastics and in antifouling paint used by shipbuilders. The intravenous injection of DBTC induces chronic pancreatic injury. Briefly, DBTC is dissolved in 100% ethanol and then mixed with glycerol. DBTC (8 mg/kg body weight) in a volume of 200 μL is then injected into the tail vein to induce chronic pancreatitis in the rat.[20] DBTC induced an acute edematous pancreatitis within 24 hours. Extensive infiltration with mononuclear cells could be observed after day 7 followed by the development of fibrosis. Parallel to the cell infiltration, an upregulation of messenger RNA-encoding collagen type I and transforming growth factor-β1 could be shown. An active inflammatory process was present until the end of the 2-month observation period. The pathological mechanism of pancreatic injury with DBTC is related to its direct toxic effect on the pancreas and its necrotic effect on the bile duct epithelium, which induces duct obstruction.[20]

L-arginine-induced chronic pancreatitis

As described earlier, a single intraperitoneal injection of high dose L-arginine (500 mg/100 mg body weight) induces severe necrotizing pancreatitis in rats. Interestingly, the long-term administration of L-arginine (350 mg/100 g body weight) for 4 weeks caused progressive degeneration of the pancreas, and only isolated single acinar cells could be seen within the fibrous connective tissue matrix contiguous with ducts, blood vessels, intrapancreatic nerves, and islets.[21] This experimental model is simple to carry out and shows features similar to those of human chronic pancreatitis, but the histological appearance is somewhat different. In this model, fibrotic tissues show progressive replacement by adipose tissue with the passage of time.[21]

CONCLUSIONS

We have reviewed several experimental models of pancreatitis. Each models have their own advantages or disadvantages. Investigator, who want to explore some aspects of pancreatitis, should be aware the characteristics of experimental model of pancreatitis and carefully choose suitable animal model for get reliable answer. Despite of researches over 100 years, there are still many questions to be answered. Genetically engineered animal would be good model to explore the pancreatitis.

Conflicts of Interest

The authors have no financial conflicts of interest.

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