Animal models of gastrointestinal and liver diseases. Animal models of acute and chronic pancreatitis

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PANCREATITIS IS THE INFLAMMATORY disease of the pancreas that causes significant morbidity and mortality throughout the world (148). There are two main types of the disease: acute pancreatitis and chronic pancreatitis.

Animal Models of Acute Pancreatitis

With ~275,000 hospitalizations in 2009 (an increase of more than twofold since 1988) (177), acute pancreatitis (AP) is the most common single gastrointestinal diagnosis, resulting in an estimated 2.6 billion dollars per year in inpatient costs (129). AP also ranks as the fifth leading cause of in-hospital deaths (85). Unfortunately, there are no effective preventive or therapeutic strategies for this disease due to the lack of a deep understanding of its pathogenesis (80).

In the United States, alcohol abuse and gallstone disease account for 70–80% of the cases of AP. Drugs, postendoscopic retrograde cholangiopancreatography (ERCP) procedures, infections, and lipid metabolic disorders can also cause AP (127). Eighty-five to 90% of AP cases are self-limiting and respond well to conservative treatment. However, the remaining 10–15% of cases with severe acute pancreatitis (SAP) are accompanied by local or systemic complications and organ failure and have a grave clinical course. Although the overall mortality in patients with AP is ~5%, the mortality of SAP is between 25 and 50% (13).

The pathophysiology of this disease is not well understood, and most of our current knowledge about the pathophysiology of this disease is from animal studies. This is partially due to the relative inaccessibility of human pancreatic tissue for examination during the early phases of pancreatitis. However, a variety of animal models have been developed to facilitate our understanding of the pathophysiology of AP. In this review, we will discuss several commonly used experimental models of AP with focus on mice and rats. Although these animal models of pancreatitis are widely used, researchers should keep in mind that no model fully recapitulates the human form of the disease. Therefore, understanding the pathophysiology and limitations of each model will guide investigators in selecting the specific model for their special purposes. Other considerations important during model selection include the cost, ease of use, reproducibility, disease severity, and clinically relevance.

It should also be noted that species, sex, and age can also affect the severity of pancreatitis. Therefore, age- and sex-matched controls should be used to offset those potential influences. As for species differences, the cholecystokinin (CCK) analog JMV-180 acts as a partial agonist in rats but a full agonist in mice (72). Unlike rodent pancreatic acinar cells, human counterparts don’t respond to CCK stimulation (71). Age-dependent effects on experimental AP in mice have also been observed (113). Male rats fed with high-fat diet have shown marked, lower pancreas Mn-superoxide dismutase activity and higher oxidative damage than females (50). These observations support the role of male hormones in the regulation of oxidative stress and pancreatitis. The evidence for female hormones in the development of pancreatitis is even more extensive. The choline-deficient, ethionine-supplemented (CDE) diet induces acute hemorrhagic pancreatic necrosis only in young female mice but not in young male mice (137).

The interplay between inflammation and the microbiome suggests that housing conditions can affect the severity and natural history of pancreatitis (181). The composition of the model’s diet can also affect inflammatory signaling (133).
Themes

G344 PANCREATITIS MODELS

Analgesics can also interfere with pancreatitis-associated inflammation; indomethacin prevents postendoscopic retrograde cholangiopancreatography pancreatitis (36). In contrast, opiate may be a cause of pancreatitis (159). Although it has been shown that caerulein-induced AP is associated with pain in experimental animals, most experiments are carried out without any pain-relieving treatment. However, in a recent study, orally administered metamizol showed no influence on the caerulein-induced AP and could be given as an analgesic to increase animal welfare in experimental models with induced AP (150).

AP is an inflammatory disease of the pancreas. The damage to the pancreas can be evaluated by measuring the serum pancreatic enzymes, such as amylase and lipase, that are released into the blood from the damaged acinar cells. There are commercially kits available that can measure these enzymes. However, the levels of these enzymes do not necessarily reflect the severity of pancreatitis. A histological examination by an experienced pathologist in a blinded manner is highly recommended to assess interstitial edema, acinar cell death (apoptosis, necrosis, and autophagy), parenchymal loss, hemorrhage, fat necrosis, inflammatory cell infiltration, and fat and fibrotic tissue replacement. However, no consensus is available regarding the criteria for scoring, and the parameters used by different investigators to evaluate the severity of AP have yet to be standardized, but most of these parameters are described in detail by Nevalainen et al. (118) and Zhang and Rouse (182). Additionally, pancreatic edema can be evaluated by whole pancreas/body weight (body wt) ratio, wet/dry pancreas ratio, or (wet–dry)/dry weight ratio.

Systematic complications involving the lung are also associated with the severity of the disease. Therefore, the histology of the lung is often included in pancreatitis research. For studies focusing on the acute respiratory distress syndrome (ARDS) in severe AP, detailed measurements of histological changes in parenchymal tissue, altered integrity of the alveolar capillary barrier, inflammation, and abnormal pulmonary function should be included. The analytical approaches, pathological features, and common measurements have been described in a recent review (3). In severe models of AP, liver/kidney malfunction can also be monitored and has been described (6, 12).

Finally, cytokine expression levels in the pancreatic tissue and serum can also be used to reflect the severity of local and systematic inflammation. It is worth emphasizing that the severity of inflammation and damage to the pancreas is not evenly distributed. Using a large piece of tissue will decrease sampling error.

Secretagogue-induced AP. In 1895, Mouret (111a) reported that excessive cholinergic stimulation was associated with the development of pancreatic acinar cell vacuolization and necrosis. Since then, pancreatitis has been reported as a complication of anticholinesterase insecticide intoxication in humans and experimental animals (34, 44). Cholinergic nerve mediation of anticholinesterase insecticide intoxication in humans and dogs (14, 46). Similarly, carbachol (a cholinergic mimetic) administration to rats produces a form of edematous pancreatitis (21, 38). However, due to systemic toxicity, the pancreatitis induced by cholinergic agonists has never been widely used in experimental settings.

Caerulein (ceruletide, also spelled as cerulein) and its structure were identified in 1967 by Australian and Italian scientists from dried skins of the Australian green tree frog (Litoria caerulea) (8). Sequencing of the peptide showed a strong resemblance in the amino acid sequence to the carboxy terminus of CCK (CCK octapeptide: Asp-Tyr[SO₃H]-Met-Gly-Trp-Met-Asp-Phe-NH₂; caerulein: pyroGlu-Gln-Asp-Tyr[SO₃H]-Thr-Gly-Trp-Met-Asp-Phe-NH₂). The seven amino acids of the COOH-terminal of caerulein are identical to those of the CCK octapeptide, except for a Thr residue that is a substitute for Met. Both peptides bear a carboxy terminal amide group, but caerulein also has a blocked amino terminal with pyrogallolamine as the initial amino acid. This blocking group reduces susceptibility to inactivation by amino peptidases (173). Additionally, sulfation of the tyrosine in caerulein is necessary for optimal physiological activity (76). In rodents, CCK receptors are present both on pancreatic acinar cells and within the neural system. Physiological levels of CCK in plasma act via stimulation of the vagal afferent pathways. In contrast, supraphysiologica plasma CCK levels act on intrapancreatic neurons and act directly to a larger extent on rodent pancreatic acini (124).

Caerulein is a potent pancreatic secretion stimulant. The dosages used for secretion studies are 1–5 ng/kg through rapid intravenous injection, 0.25–1 ng·kg⁻¹·min⁻¹ through intravenous infusion, and 50–100 ng/kg through subcutaneous injection (16, 33). At higher doses (20 ng·kg⁻¹·min⁻¹), caerulein causes zymogen activation, large vacuole accumulation, endoplasmic reticulum (ER) dilation and segmentation, and mitochondrial swelling (155). In 1977, Lampel and Kern (84) discovered that excessive doses (5 µg·kg⁻¹·h⁻¹) of caerulein-induced acute interstitial pancreatitis in rats.

Supramaximal doses of caerulein induce a significant increase in serum pancreatic enzymes, pancreatic interstitial edema, and inflammatory cell infiltration. It has been tested in rats, mice, dogs, and hamsters, and all of the animals survived the induction of pancreatitis (172). The changes caused by caerulein infusion occur within 15 min after infusion. Cytoplasmic vacuoles are the earliest histological alterations. As the pancreatitis progresses, these vacuoles increase to an enormous size. Electron microscopy showed both intact and degenerative granules inside the vacuoles. Interstitial inflammation and acinar cell necrosis were prominent 6 h after injection and reached a maximum after 12 h. These changes usually resolved within a week. During the regression of pancreatitis, focal atrophy was a remarkable histological finding (119).

The mechanisms of secretagogue-induced AP have been extensively exploited over the past few decades. For in vitro studies, primary pancreatic acini can be isolated from mouse and rat pancrea by mechanical dissection followed by purified collagenase digestion (174). These pancreatic acini can then be treated with CCK or other secretagogues. CCK regulates a complex array of cellular functions in pancreatic acinar cells through its G protein-coupled receptor, which activates a variety of intracellular signaling mechanisms. CCK couples through heterotrimeric G proteins to activate phospholipase C, increasing inositol trisphosphate and releasing intracellular Ca²⁺. This pathway and protein kinase C activation leads to the secretion of digestive enzymes by exocytosis. CCK also activates Nuclear Factor of Activated T cells (NFATs), ERKs,
JNKs, and p38 MAPK, which are involved in secretion and growth (58, 175). In addition to Gq, CCK receptors also activate G12/13, Rho, and Rac to regulate the cytoskeleton and secretion (17, 88).

CCK elicits a concentration-dependent effect on pancreatic enzyme secretion. At low physiological concentrations, CCK stimulates pancreatic acinar cell secretion. However, at pathophysiological concentrations, the secretion of the pancreas is inhibited due to the altered influx of intracellular calcium, diminished pancreatic enzyme secretion into the duct, pathological basal-lateral secretion/leakage of active enzymes, premature intracellular zymogen activation, actin cytoskeleton reorganization, ER stress, activation of NF-κB and apoptosis, and autophagy. Increased vascular permeability and increased hydrostatic pressure may contribute to the extensive edema associated with pancreatitis. Cytokine storms and active pancreatic enzymes lead to a systemic inflammatory response syndrome (SIRS), which includes extrapancreatic damage, such as pancreatitis-related lung injury (18, 73, 98, 103, 112, 142, 157).

In addition to the direct effect of CCK on acinar cells, CCK also acts via the vagal afferent pathways to mediate pancreatic secretion. Atropine, a muscarinic receptor antagonist, completely abolishes pancreatic enzyme responses to physiological doses of CCK in rats, which suggests that CCK acts on a presynaptic site along the cholinergic pathway (95). In healthy human subjects, CCK infusions produce plasma CCK levels similar to those seen postprandially and stimulate pancreatic secretion by an atropine-sensitive pathway. These observations suggest that both in experimental animals and in humans cholinergic neural pathways, rather than pancreatic acini, represent the primary targets in which CCK acts to stimulate pancreatic enzyme secretion (2). Cholinergic stimulation of pancreatic amylase secretion is mediated in mice by a mixture of M1 and M3 muscarinic acetylcholine receptors. Like CCK receptors, these receptors are selectively coupled to G proteins of the Gq family, which mediate the breakdown of phosphatidyl inositol lipids (47). Similarly, muscarinic-receptor agonists stimulate trypsinogen and NF-κB activation, two key signaling pathways in the pathogenesis of pancreatitis. Interestingly, ethanol enhances carbachol-induced protease activation and accelerates Ca²⁺ waves in isolated rat pancreatic acini. These observations support the key roles of the cholinergic system in the mechanisms of alcoholic pancreatitis (59, 102, 123, 185). The cholinergic signaling may be of particular importance in humans because there are no functional CCK receptors on human pancreatic acinar cells (71).

There are great variations among the protocols used in different laboratories. Although jugular or tail vein infusion was originally used, it was soon replaced by intraperitoneal injection due to its convenience (119). Typically, caerulein (50 μg/kg body wt; dissolved in phosphate-buffered saline or 0.9% saline in a volume of 100 μl) is injected intraperitoneally every hour for 8–12 injections. The pancreata are usually harvested 1 h after the last injection or 24 h after the first injection. For regeneration studies, the pancreata can be collected after 2–7 days. For early signaling studies, the pancreata can be removed at a desired time after a single dose of injected caerulein. Caerulein induces parallel activation of both NF-κB and trypsinogen in the pancreas in vivo as early as 15 min after injection (64). Of note, caerulein is usually provided in 1-mg aliquot powder, and the solubility of caerulein from different providers may vary. If the powder does not completely dissolve, then it will result in a low concentration of caerulein and may fail to induce pancreatitis.

The secretagogue overstimulation model of experimental pancreatitis is so far the most commonly used AP model. The model has several advantages, including noninvasiveness, high reproducibility, and applicability in multiple species. In addition, the in vivo experiment can be paralleled with in vitro acinar cell studies, which makes this model extremely useful for the investigation of intracellular signal transduction events, protease activation cascades, and cell death pathways involved in the early phase of AP. Moreover, this is a great model for the investigation of recovery and regeneration of damaged tissue after the toxic substances have been discontinued. However, this model has several major disadvantages. Even with maximum dosage of caerulein, only mild, self-limited AP but not severe necrotizing pancreatitis is recapitulated, which limits its use in the study of severe pancreatitis, which carries the highest morbidity and mortality. In addition, secretagogue overstimulation is not a common cause of AP in humans, although AP cases have been reported in patients who accidentally received high concentrations of secretagogue (14), were exposed to cholinesterase inhibitors such as insecticides (149), or were exposed to Trinidadian scorpion toxin (14). Moreover, major differences exist between the human and rodent pancreas: CCK plays a major role in regulating exocrine pancreatic secretion in rodents; however, human pancreatic acinar cells are mostly regulated by cholinergic pathways that involve neurogenic CCK stimulation (71, 124).

Basic amino acid-induced AP. In an effort to study the effects of arginine on normal tissues, Mizunuma et al. (110) unexpectedly found that single intraperitoneal injection of L-arginine (500 mg/100 g body wt) in rats selectively destroyed pancreatic acinar cells. The model was further studied by Tani et al. (154) in 1990. From then on, several groups have used this rat model. However, with this protocol the same dose of L-arginine failed to induce reproducible pancreatitis in either Balb/c or C57Bl/6 mice. In 2007, Saluja and colleagues (29) developed a mouse model of acute necrotizing pancreatitis using intraperitoneal applications of L-arginine hydrochloride solution (8% in normal saline, pH 7.0).

The dosage of L-arginine on AP development is species dependent. In mice, L-arginine has no effect on pancreatic histology when administered at doses lower than 4 g/kg and when administered twice (1 h apart). However, in rats, intraperitoneal administration of 2 g/kg L-lysine induced severe acute necrotizing pancreatitis (20).

In rats given single intraperitoneal injections of L-arginine at 500 mg/100 g body wt, serum levels of amylase, lipase, and anionic trypsin reached their peaks after 12–24 h but returned to normal levels after 24–48 h. Histological examinations revealed a number of small vesicles within acinar cells after 6 h, which were identified as markedly swollen mitochondria by electron microscopy. Interstitial edema appeared after 12 h, and acinar cell necrosis was seen after 24 h. The extent and severity of necrotic changes of pancreatic exocrine tissue with inflammatory cell infiltration was maximal after 72 h. After 7 days without treatment, pancreatic acinar cells began to regenerate, and pancreatic architecture appeared almost normal after 14 days without treatment (154). In this model, pancreatic...
acinar cells are visible. However, pancreatic islet cells remain unaffected by L-arginine treatment (29).

Although the exact mechanisms of L-arginine pancreatitis are not clear, the imbalance of amino acids and the subsequent decrease of protein synthesis, the derangement of transamination and the urea cycle, and metabolic acidosis in the acinar cells may all be involved. L-Arginine metabolites are also involved in pancreatitis development as high doses of L-ornithine, a downstream product of L-arginine by L-arginase, have also been reported to cause AP (136). Partial inhibition of L-arginase ameliorates L-arginine-induced pancreatitis (19). Oxidative stress may also contribute to L-arginine pancreatitis because reducing oxidative stress ameliorates pancreatic injury in this model of pancreatitis (61, 62). Additionally, hypersecretion of protein, RNA, and phospholipid metabolism (100) may also be involved in the development of this disease (151, 153). These effects seem to be L-arginine specific as D-arginine, L-lysine, L-alanine, and glycine failed to elicit AP.

The L-arginine model has been modified to a single injection with varying results. Doses higher than 500 mg/100 g body wt can induce death within a few hours (154). A single dose of 500 mg/100 g causes necrosis in up to 70–80% of the pancreatic acinar cells within 3 days, which increases to 90% after three further doses over 10 days (154). Splitting the dose to two injections can also be used to delay the onset of AP (169). Single injection carries a higher mortality than double injection, and therefore double injection models are more commonly used. In brief, a sterile L-arginine solution (8% in PBS or normal saline, pH 7) is administered intraperitoneally to nonfasted mice at a dose of 400 mg/100 g body wt or to rats at a dose of 250 mg/100 g body wt. One hour later, a second dose of L-arginine is administered.

L-Arginine-induced AP provides a model for necrotizing pancreatitis that carries a high morbidity and mortality. It is highly reproducible, and the severity of pancreatic acinar necrosis can be controlled by regulating the dose and time of L-arginine. However, it is unlikely that human AP is caused by basic amino acid overdose.

CDE diet-induced AP. Pancreatic damage caused by low-choline diets was originally reported in 1937 (54). In 1950, ethionine was first reported to induce AP in rats by Farber and Popper (40) and Goldberg et al. (49). Since then, ethionine-induced pancreatitis has been shown to occur in many other species of experimental animals, including mice, hamsters, cats, dogs, and monkeys (22, 30, 166). Ethionine-induced pancreatitis is characterized mainly by local necrosis and atrophy with acinar cell regeneration. In 1975, Lombardi and colleagues (100, 138) reported that female mice fed with the CDE diet (a choline-deficient diet enriched with 0.5% ethionine, a derivative of methionine) developed severe necrotizing pancreatitis and had a mortality rate of 100% after 4 days. The onset of CDE diet-induced AP is variable, but it usually takes 2–3 days to develop. This form of pancreatitis is characterized by massive fat necrosis of the exocrine parenchyma, intense hemorrhaging, and an inflammatory reaction of the stroma throughout the peritoneal cavity (99, 100, 120, 138). The earliest changes are increased zymogen granules in pancreatic acinar cells. Pancreatic inflammation and necrosis occur before activated proteolytic enzymes can be detected within the pancreas. The necrosis of acinar cells develops 48 h after treatment, and hemorrhaging starts 60 h after initiation of the diet. In the animals who survived the AP, their pancreatic function started to recover after 2–3 wk, and morphological resolution usually took 4–6 wk.

The exact mechanisms of CDE on AP are unclear. However, the obvious sex differences in this model suggest that estrogen may play a role in the pathogenesis of AP and have been evidenced by the fact that male mice pretreated with estrogen can also develop AP on CDE diet. Additionally, zymogen-granule secretion blockage and intraparenchymal trypsinogen activation can be a result of a synergistic action of choline deficiency with the basic toxicity of ethionine toward the acinar cells. Ethionine may be toxic to the pancreas by interfering with RNA, protein, and phospholipid metabolism (100). Autophagy and crinophagy have also been suggested to be involved in the vacuole formation.

The severity of the CDE AP model will vary depending on the sex, age, and weight of the animals. Young, female mice are prone to have more severe cases compared with older, male mice. Generally, young female CD-1 mice (or NMR1, or Swiss Webster mice; 4–6 wk old, 10–14 g) are used for the CDE AP model. Typically there are two approaches to generate this model. In one approach, the animals are fed regularly with choline-deficient diet throughout the day and switched to the CDE diet. The mortality of the animals depends on the length of time the animal is on the CDE diet: 33 h renders 10–20% mortality, 48 h increases the mortality to 40–70%, 66 h is associated with 55–75% mortality, and if the diet is administered for 72 h, then there will be a mortality of over 80%. Another approach involves animal starvation for 1 day followed by feeding with the CDE diet at 3 g per mouse per day for 1–5 days depending on the desired severity of AP. In this approach, the animals tend to eat more of the CDE diet during the first 24 h and develop AP faster because they have been starved for 24 h (120).

This model is a noninvasive AP model. Despite the differences in pathogenesis of the pancreatitis induced in this model compared with the human disease, the gross and histological appearance of the pancreatic and periampullary inflammation and the clinical and biochemical course of the diet-induced pancreatitis resemble the human disease. Both the model and the human disease share several pathophysiological features, including necrosis, systemic hypoxia, and hemococoncentration. Therefore, the model may be suitable for studying these pathophysiological aspects of this disease. The severity and mortality of the model can be modified by changing the duration of CDE diet administration; for example, all of the mice fed with the CDE diet for 5 days died of hemorrhagic pancreatitis;
However, when the CDE diet was given for only 1 day, the incidence of fatal hemorrhagic pancreatitis was reduced to 55–65% (120).

Several pitfalls and problems have to be considered to obtain valuable data. Due to the mortality associated with the amount of the CDE diet ingested, the CDE diet implementation requires careful monitoring of dietary intake and animal status. It is also important to use large numbers of animals in each experimental group. Young mice are affected more severely than adult mice and females more than males (101). Thereby age- and sex-matched animals should be used for homogeneity and reproducibility of CDE diet-induced AP. Another point to remember is that liver damage and hypoglycemia are also observed in this model. Therefore, this model is not ideal for studying multiple organ distress syndromes because the CDE diet can trigger those syndromes by mechanisms that are unrelated to the severity of AP. The high cost of the diet also discourages the use of this model (120). Because of these limitations, this model has been rarely used in recent years.

**Retrograde ductal infusion-induced AP.** Biliary pancreatitis is one of the most common forms of AP. One theory is that biliary obstruction permits the reflux of bile into the pancreatic duct. The first experimental, retrograde ductal infusion-induced AP model was established in 1856 by Bernard (15), who injected bile and olive oil into a canine pancreas through the ampulla of Vater. Since then, various bile salts have been reported to induce AP in different species. Ductal infusion of sodium glycodeoxycholic acid dose dependently induces edematous pancreatitis and necrotizing pancreatitis in rats (156). An infusion containing a combination of enterokinin with sodium glycodeoxycholic induced necrotic pancreatitis with systemic complications and rapid mortality (156). In 1992, Schmidt et al. (145) modified the rat duct infusion model by using the combined actions of very low concentrations of glycodeoxycholic acid (5–10 mM) administered via ductal infusion and caerulein (5 μg·kg<sup>−1</sup>·h<sup>−1</sup> for 6 h) administered intravenously. This model features a moderate onset of moderate injury throughout the pancreas that lasts 24 h and provides the potential for modulating disease severity (41). Recently, Lauk- karinen et al. (87) established a retrograde ductal infusion-induced AP model in mice by infusing sodium taurocholate into the mouse pancreatic duct. In the head of the pancreas, evidence of pancreatitis was observed 12–24 h after infusion of 20–50 μL of 2–5% sodium taurocholate. The damage in the pancreas was concentration and volume dependent. There was no lethality found. In contrast, in a separate study using mice (176), 2–5% sodium taurocholate solution was injected at a volume of 2 mL/kg (50 μL/mouse). All of the animals receiving 2 or 3% sodium taurocholate survived the experiment. Animals receiving 4 and 5% sodium taurocholate showed a significant increase of fatty tissue necrosis when compared with animals receiving the 2% solution. Death within the first 24 h occurred in 10% of the animals receiving the 4% solution. And death rate reached 60% when 5% taurocholate solution was used.

Histological changes of the pancreas after 24 h of treatment include large areas of acinar cell necrosis adjacent to morphologically unaltered acinar lobes. Leukocyte infiltration, fatty-tissue necrosis, and hemorrhage are typical. Taurocholate infusion predominantly affects the pancreatic head. The pancreatic tail usually presents with extensive edema. Surprisingly, despite the marked lethality associated with the 5% sodium taurocholate treatment, no obvious pulmonary pathology was detected after 24 h postinduction (176).

Bile salts exert detergent properties and primarily aid in the absorption of fat and fat-soluble vitamins (65). It was initially believed that pancreatic injury resulted from the detergent properties of bile acids. Currently, bile acids are considered crucial agonists that interact with several proteins, including the nuclear receptor farnesoid X receptor (FXR) and G protein-coupled bile acid receptor-1 (Gpbar1) to regulate many cellular functions (39, 48). Bile acids can act on both duct cells and acinar cells of the exocrine pancreas. Bile acids have dual effects on the ductal secretion, and at low concentrations they induce a dose-dependent elevation of intracellular Ca<sup>2+</sup> concentration via an inositol 1,4,5-triphosphate receptor and a phospholipase C-mediated pathway. This may help wash out the toxic bile acids and thus protect the acinar cells. At high concentrations, bile acids induce a toxic, sustained intracellular Ca<sup>2+</sup> concentration and decrease intracellular ATP, which inhibits all of the acid-base transporters. Pathological Ca<sup>2+</sup>-dependent trypsinogen and calcineurin activation causes cell death in isolated pancreatic acinar cells (112). Loss of the flux defense mechanism may also lead to high concentrations of bile acids to reach the acinar cells. These effects at least in part are mediated by Gpbar1. Genetic deletion of Gpbar1 significantly reduces biliary but not secretagogue-induced experimental AP, suggesting its role in the pathophysiology of the biliary pancreatitis (130).

One of the most commonly used retrograde ductal-infusion protocols in rats was described by Aho et al. (5). First, a midline laparotomy is performed, the biliopancreatic duct is then cannulated, and fluid (saline or saline containing bile acid and blue dye) is infused. Flow rates higher than 10 μL/min should be avoided to minimize any artifacts or injury due to the rapid increase in intrapancreatic pressure. Detailed methods for experimental acute biliary pancreatitis in mice induced by retrograde infusion of bile acids have been published by Perides et al. (132).

Biliary AP accounts for 30–50% of all clinical cases of AP. This model uses clinically relevant etiological insults of acute biliary pancreatitis, and its severity can be manipulated by altering the concentration, volume, and infusion pressure of the injected bile acid. This model is useful for mechanistic studies employing genetically modified mouse strains. The disadvantage is that it is technically challenging to control the constant pressure when retrograding ductal infusion, which is critical for determining the severity of the induced pancreatitis. Pumps may be needed to control the constant low pressure of infusion to produce a standard degree of pancreatic injury and to avoid high perfusion pressure-induced artifact (87).

Recently, Husain and coworkers (75) developed another clinically relevant mouse model of AP. In this model, the radiocontrast agent iohexol was injected to the common bile duct and pancreatic duct of Swiss Webster mice by retrograde infusion. Iohexol infusion causes acute damage and inflammation in the pancreas. Mechanistically, radiocontrast agents cause a pancreatic inflammatory response through the activation of NF-kB, calcium signaling, and calcineurin pathways. These signaling pathways seem to be pancreatic acinar cell specific because they were not elicited in HEK293 or COS7 cells. Calcineurin deficiency or inhibitors improved the pan-
Duct-ligation induced AP. This model is based on the theory that duct obstruction will block pancreatic fluid and enzyme flow and therefore lead to AP development. Initially attempted in the early 19th century (81), duct ligation has been used in various animals including dogs, rabbits, opossums, rats, and mice. Opossums have a pancreatic main duct that drains into a long common bile duct, and both enter the duodenum at the papilla. The extraduodenal common bile duct and the pancreatic duct are easily dissected in this animal and as a result make it easier to study the effects of ligation of specific ducts on the pathogenesis of AP. Ligation of the pancreas duct or common bile duct in opossum induced hemorrhagic AP with a 14-day mortality of 100% (147). However, most other laboratory animals do not develop AP with surgical ligation of the pancreatic duct alone, but long-term ligation causes chronic atrophy of the exocrine pancreas (122, 167). In mice, the combination of bile infusion into the pancreas followed by bile duct ligation also causes a more severe, necrotizing pancreatitis (89). A similar level of severity seen after obstructing the pancreatic duct adjacent to duodenum (which also blocks the bile duct) compared with ligation of both pancreatic duct and bile duct adjacent to liver suggest that bile reflux may not be necessary to induce AP (92). In another study in mice, the pancreatic duct was blocked by placing a small metal clip across the lower end of the duct. A suture was incorporated within the clip during its placement for reversing biliary obstruction as needed (82). Combining duct ligation with both secretory stimulation or minimized arterial blood produced a more severe form of AP (135).

Pancreatic duct obstruction rapidly changes the physiological response of the exocrine pancreas to a calcium signaling pattern that has been associated with premature digestive enzyme activation and the onset of pancreatitis (111). It is postulated that intrapancreatic digestive enzyme activation accounts for the major pathology of this model. Altered intracellular targeting of endocytosed proteases might be one mechanism by which digestivezymogens reach an intracellular compartment where premature activation can occur (93).

The duct ligation-induced model closely mimics gallstone-induced AP (1). It avoids the use of agents at nonphysiologically relevant concentration and dosage that could produce unwanted systemic effects. However, the complexity, technical difficulty, and limited reproducibility have made the duct ligation model less popular for investigating AP. Duodenal wall necrosis and pancreatic peritoneal sepsis often add complexity to the model. Furthermore, the severity of AP varies depending on the species and duration of obstruction (23, 122). There are also species-dependent effects in this model. In duct-ligated rats, apoptosis is the main mechanism of cell death. By contrast, necrosis is the predominant mechanism in duct-ligated opossums (56, 79).

Alcohol-related AP. Although alcoholism is one of the major causes of human pancreatitis in the United States, a continuous intragastric infusion of ethanol to rats over several weeks produced only ethanol-induced liver injury, not alcohol-induced pancreatitis. The failure to produce alcoholic pancreatitis in animals suggests that the provision of ethanol may only increase the predisposition to pancreatitis. In 1999, Pandol et al. (126) found that an ethanol diet sensitized rats to the pancreatitis caused by CCK-8. After intragastric feeding with either a control or an ethanol diet for 2 or 6 wk, rats were then infused for 6 h with either saline or CCK-8 at a dose of 3,000 pmol·kg⁻¹·h⁻¹, which by itself did not induce pancreatitis. All measures of pancreatitis, as well as NF-κB activity and cytokines were significantly increased only in rats treated with ethanol plus CCK-8 (126). In a later study, rats were fed with control and ethanol-containing Lieber-DeCarli diets (discussed in detail below: Alcohol-related CP models) for 6 wk followed by four hourly intraperitoneal injections of caerulein at 0.5 μg/kg. Caerulein alone induced only minor pathological changes in control-fed rats. In contrast, in ethanol-fed rats, caerulein induced marked acinar cell vacuolization, dilation of the ER, and occasional patchy cellular necrosis (102).

Both oxidative and nonoxidative metabolisms of alcohol (OME and NOME, respectively) mechanisms are used to degrade alcohol in the exocrine pancreas. NOME combines ethanol with fatty acids to yield lipophilic fatty acid ethyl esters (FAEEs) via diverse FAEE synthase enzymes (55). FAEEs can induce high sustained toxic calcium concentrations in pancreatic acinar cells and lead to acinar necrosis (27). To test the effects of FAEE in the initiation of AP, adult CD1 mice received two intraperitoneal injections of ethanol (1.35 g/kg) and POA (palmitoleic acid, a monounsaturated fatty acid, 150 mg/kg) at 1-h intervals. Two hundred microliters of normal saline were injected immediately prior to ethanol/POA injections to avoid local damage by ethanol to peritoneal organs at the injection site. Mice received either saline, ethanol (1.35 g/kg), or POA (150 mg/kg) were used as controls (67). At 24 h after application, the combination of ethanol and POA induced pancreatic damages including extensive acinar cell edema, neutrophil infiltration, and necrosis. The combination of ethanol/POA also elicited alveolar membrane thickening and inflammatory cell infiltration in the lung but damages in the liver, kidney, or heart are minimal (67). A similar phenotype was achieved with the use of C57BL/6J mice (170). Mice are more tolerant to the treatment after one instead of two doses of treatment (107).

Coxsackie B virus-induced AP. A wide variety of infections have been associated with AP. These infections include viruses (mumps, coxsackie, hepatitis B, cytomegalovirus, varicella-zoster virus, herpes simplex virus), bacteria (Mycoplasma, Legionella, Leptospira, Salmonella), fungi (Aspergillus), and parasites (Toxoplasma, Cryptosporidium, Ascaris) (128). Among these factors, coxsackie B virus-induced AP has been well documented and studied (24, 60, 139).

Coxsackie virus-type B virus is a single-stranded RNA picornavirus with six identified serotypes (serotypes 1–6). Mice infected with coxsackie viruses B1, B3, B4, or B5 produce a severe form of pancreatitis consisting of the degeneration of acinar cells, loss of zymogen granules, infiltration of mononuclear and plasma cells, and the replacement of exocrine tissue with fatty tissue. In contrast, coxsackie viruses B2 and B6 do not cause these changes. Coxackie viruses B3 and B4 exhibit more rapid action in the tissue and more severe lesions than B1 and B5. In contrast, none of the coxsackie B viruses examined elicited detectable microscopic changes in the islets of Langerhans (86). In newborn mice infected with coxsackie
B4 virus by intraperitoneal inoculation for 1–2 days, cyto-
ecrosis consistent with a picornaviral infection was observed
(60). Mice with coxackievirus B3 infection developed necro-
tizing AP. This form of pancreatitis eventually led to complete
atrophy of the exocrine pancreas with the islets of Langerhans
and pancreatic ducts spared (165). These mice also developed
focal myocarditis (51). The viral infection also interacted with
other modifying factors. For example, it was observed that
infection of the virulent coxackievirus B3 strain 28 in com-
bination with alcohol feeding resulted in a more severe form of
pancreatitis. Furthermore, infection with the avirulent strain
(GA) plus alcohol feeding caused severe pancreatitis, whereas
the infection alone did not result in any obvious pathological
effects in the pancreas (70). Alcohol also impaired pancreatic
regeneration following viral-induced injury in a dose- and
duration-dependent fashion (25).

Animal Models of Chronic Pancreatitis

Progressive inflammation of the pancreas leads to chronic
pancreatitis (CP), which is defined by the loss of parenchymal
(exocrine and endocrine) cells, inflammatory cell infiltration,
fat replacement, stellate cell activation and fibrosis, calcifica-
tion, and nerve enlargement. Clinical manifestations include
pain, maldigestion, and possible diabetes. Although pain is
common, exocrine insufficiency occurs late in the course of
the disease because the pancreas has a great functional reserve.
Steatorrhea occurs only when lipase secretion is reduced to less
than 10% of normal (162). In the absence of established
diagnostic criteria, early diagnosis of human CP is challenging
and often based on a combination of clinical presentation,
imagining results, and pancreatic function test results (35, 105).
There is also no consensus on the criteria for evaluating animal
models of CP. Because the purpose of pancreatitis research is
to eliminate the inflammatory response, prevent fatty/fibrotic
replacement, and preserve both exocrine and endocrine pan-
creatic functions, the parameters used to evaluate CP include
pancreatic weight, blood insulin/glucose levels, pancreatic am-
lyase content, inflammatory cell including macrophage and
lymphocyte infiltrations, α-SMA expression (a marker for
early stellate cell activation), and areas of fatty/fibrotic replace-
ment (45, 69, 74, 140, 141, 183).

The pathogenesis of CP is poorly understood. Accumulated
evidence suggests that the incidence of CP is increasing (77).
Alcohol is the major cause of CP in the Western world. Other
etiologies include smoking, obstructive lesions, other toxic
agents, and genetic factors (178). Ethanol has both direct and
indirect toxic effects on the integrity of pancreatic acinar cells
that are mediated by the ethanol metabolite acetaldehyde (7).
Oxidative stress caused by ethanol or nicotine can lead to the
peroxidation of the lipid bilayer of the cell membrane, which
consecutively disintegrates the membrane (108). Different eti-
ologies may have different pathogenic mechanisms, and di-
verse signaling leads to various phenotypic changes. For ex-
ample, prolonged activity of NF-κB results in inflammatory
cell infiltration, activation of stellate cells, loss of acinar cells,
and fibrosis, which are characteristics of CP (66). Ras activa-
tion leads to acinar cell senescence and generates inflammation
and massive fibrosis resembling the histological features of CP
(28, 74, 97). In contrast, the primary response to intracellular

trypsin activity is the rapid induction of acinar cell death via
apoptosis and causes fat replacement in the pancreas (45).

Pancreatic fibrosis is a histopathological feature of CP. It is
now recognized that pancreatic fibrosis is mediated by the
activation of pancreatic stellate cells (PSCs) that normally
reside in the periacinar region of the pancreas in a quiescent
state (9). The mechanisms of PSC activation have been studied
and reviewed previously (11). It seems that fibrosis is an active
process mediated by the activation of PSCs. Acinar cell apo-
ptosis that is induced by intracellular trypsin caused extensive
fat replacement (45). In contrast, Ras-induced acinar cell se-
nescence led to a dramatic desmoplasic reaction in the pan-
creas (74).

In humans, acute and recurrent AP can develop into CP.
These diseases share similar genetic and environmental caus-
tive factors. Therefore, AP, recurrent AP, and CP are now
considered as a disease continuum (171). Indeed, many re-
searchers have tried to establish CP models by using modified
experimental protocols that cause AP.

Models based on repetitive induction of AP. In caerulein-
induced AP, the pancreas will recover from a single episode of
cerulein challenge within a week. Expression of TGF-β1, a
key profibrogenic factor, is increased in acinar and stromal
cells of the rat pancreas in this model, indicating that repetitive
treatment with caerulein may cause fibrosis, a key feature of
CP (53). Indeed, repeated caerulein insult could induce exo-
crine deficiency, fibrosis, and diabetes (115). As in AP models,
this is the most commonly used and reproducible model for
CP. The procedures vary in different studies and can be used in
combination with many other insults. In one early study, rats
were treated with water immersion stress for 5 h and two
intraperitoneal injections of caerulein (20 μg/kg body wt) once
a week for 16 wk. Pancreatic atrophy, marked fibrosis, fatty
changes, and destruction of the lobular architecture were demon-
strated microscopically; diabetes was also present (52).
Acute pancreatic injury induced in mice by twice-weekly
cerulein treatment (50 μg·kg⁻¹·h⁻¹ × 6 h) for 10 wk in-
creased procollagen expression and progressive accumulation
of the extracellular matrix surrounding acinar units and in
interlobular spaces. Atrophy, transdifferentiation of acinar
units to ductlike tubular complexes, and dilatation of intra-
acinar lumina also developed (117). A more closely spaced
treatment strategy in which AP was induced three times weekly
instead of twice weekly produced an even more rapid accumu-
lation of periacinar fibrosis and altered acinar architecture 6 wk
after the initial AP induction (116, 158). After the insults were
stopped, the pancreatic fibrosis mostly resolved in 3–6 wk,
suggesting that the processes responsible for matrix resolution
are potentively active in the pancreas (116). Additionally, the
addition of lipopolysaccharide (LPS) increased the amount of
fibrosis (121). This may be because pancreatic stellate cells
express a variety of Toll-like receptors (TLRs) and respond to
TLR ligands (e.g., LPS), leading to the activation of signaling
pathways and proinflammatory responses (109).

Cyclosporin is a drug that binds to cyclophilin to inhibit
calcineurin and increases TGF-β expression. Although cyclo-
sporin accumulates at high concentrations in the pancreas, it
only produces minor morphological or functional derange-
ments. However, the combination of cyclosporin and caerulein
exacerbates the chronic inflammatory response in the pancreas
(161), but the doses and duration of caerulein treatment and the

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timing of tissue collection seem to be important in determining the phenotype observed. In one study, pancreatitis was induced nine times at intervals of ~20 days; 3 days after the last injection of caerulein, inflammation was still observed. However, 6 wk later, the pancreas fully recovered (37). Using higher frequencies of caerulein applications will likely lead to more a dramatic formation of pancreatic fibrosis.

Serial intraperitoneal injections of arginine with initial dose of 500 mg/100 g body wt followed by three injections at 250 mg/100 g over 10 days was also used to produce CP. After 24 h the pancreas had severe edematous AP. By day 5 there was up to 90% acinar destruction with adipose tissue replacement, but ductal and islet cells appeared undamaged. These changes were still present 6 mo after injection (31). In another study, male rats were intraperitoneally injected daily (350 mg/100 g body wt) with l-arginine from 1 to 4 wk. At week 1, light microscopy revealed areas of focal acinar cell degeneration. At the end of week 2, acinar necrosis was evident throughout most pancreatic lobules. At week 4, only isolated, single acinar cells remained within a fibrous connective tissue matrix contiguous with ducts, blood vessels, intrapancreatic nerves, and islets (169). Instead of fibrosis, Yamaguchi et al. (180) showed that 85 to 90% of the acinar tissue was replaced by fatty tissue and dilated pancreatic ducts on day 54 after the first intraperitoneal arginine injection.

Mice fed with an intermittent CDE diet (3 days of CDE diet alternating with 3 days of normal diet) for a prolonged period of 24 wk can develop some histological features of CP. The fibrosis in this model is mild (69).

In mice and rats, complete pancreatic duct obstruction mainly results in atrophy of the organ and does not lead to the typical morphology of CP. Several months after pancreatic duct ligation, there is intralobular fatty replacement of the exocrine pancreas (168). Therefore, duct ligation alone does not cause typical CP. However, complete pancreatic duct obstruction and daily caerulein hyperstimulation caused large amounts of interstitial collagen expression after 72 h (114). Recently, a modified-CP model by branch duct ligation in combination with caerulein administration has been developed and could be a valuable model (146). In this study, CP was induced by ligation of the pancreatic duct at the junction between the gastric and the duodenal lobe sparing the bile duct and its concomitant artery. These animals then received a single injection of caerulein (50 μg/kg body wt) 2 days after duct ligation. Severe necrotizing pancreatitis developed in the ligated part of the pancreas 3 days after surgery. Model-associated mortality ranged from 10 to 15% and always occurred within 48 h of caerulein injection. Areas of pancreatic necrosis were replaced by fibrotic and fatty tissues, and maximal fibrosis was detected after 21 days.

The mortality rate is usually high in acute hemorrhagic pancreatitis that is induced by retrograde infusion of sodium taurocholate into the pancreatic duct system of the rats. In animals surviving 72 h, there was marked acinar atrophy and pancreatic fibrosis (5).

However, with a single retrograde intraductal infusion of 40 μL/100 g body wt of 3% sodium taurocholate, the pancreas appeared to be histologically normal 42 days after intraductal infusion (180). The volume, concentration, pressure, and duration may be factors that are complicated in these phenotypic changes.

Alcohol-related CP models. In 1967, Maki et al. (106) studied the influence of the oral administration of alcohol on the rat pancreas and the interaction of alcohol with various dietary compositions. Alcohol with a concentration of 15% was added to the drinking water of the study animals for 2 mo. The alcohol intake caused increased serum amylase levels, pancreatic edema, and vacuolization of acinar cells, but fat necrosis or pancreatic necrosis was not noted. Interestingly, the rats that were fed on a high-carbohydrate diet showed minimal changes in the pancreas compared with those fed on high-protein and high-fat diets and with those fed on low-protein and high-fat diets. In 1971, Sarles et al. (144) fed rats with 20% ethanol for 20–30 mo. The average daily consumption of alcohol was 4 g/rat. More than half of the animals developed pancreatic lesions similar to those of human CP. The pathological changes included reduced acini, duct multiplication, protein plugs, sometimes calcified ducts, and sclerosis. Beta-cell adenomata of the islets of Langerhans were also observed in four of the rats exposed to ethanol.

Currently, the most popular protocol used to imitate the effects of alcohol on various organs was developed by Lieber and DeCarli in 1975 for alcohol liver injury (96). The feeding regime was based on the supplementation of ethanol into a liquid diet (referred as Lieber-DeCarli diet), and it allows an increased total alcohol intake. Isocaloric substitution of carbohydrates by ethanol (36% of total calories in rats) resulted in the production of fatty liver disease, alcoholic hepatitis, and eventually cirrhosis. This diet leads to severe organ damage in the liver but pancreatic morphology changes are mild. Rather than CP, only mild functional insufficiency develops in most cases. These observations correlate with the clinical finding that only a minority (less than 10%) of alcoholics ever develop clinical CP (94). Therefore, alcohol alone is a very weak inducer of CP, and other factors are necessary for the onset of CP. However, the Lieber-DeCarli administration causes a number of changes that may predispose the gland to damage. These changes include an increased concentration of digestive enzymes and the lysosomal enzyme cathepsin B, which is important in intracellular trypsinogen activation. It is believed that alcohol “sensitizes” or “primes” the pancreas to pancreatitis; current studies are focused on the mechanisms responsible for the sensitizing effects of alcohol, and Lieber-DeCarli diet is usually used in combination with many other factors (genetic, environmental, or dietary) to induce CP (10, 125).

The pancreata from rats fed ethanol for 9–12 mo are more susceptible to caerulein-induced activation of chymotrypsinogen than the pancreata from pair-fed control animals (134). The combination of alcohol feeding with caerulein injections exacerbates pancreatitis with increased fibrosis and loss of parenchyma. Additionally, calcifications indicating severe CP can be observed. Although pancreatic function was not directly tested, there was evidence that digestive enzyme synthesis is reduced after chronic ethanol feeding (32, 131).

Combining LPS and the alcohol model can be adopted because of its potential clinical relevance. In one study, alcohol-fed rats receiving a single LPS injection displayed more severe acinar vacuolization, acinar cell necrosis, inflammatory infiltrates, and hemorrhaging. Negligible lesions were found in the control diet-fed plus LPS group. These lesions mimic some of the features of AP (164). When the rats fed Lieber-DeCarli liquid diets for 10 wk were challenged with three repeated
doses of LPS (3 mg/kg intravenously per week for 3 wk), significantly greater pancreatic injury and pancreatic fibrosis were seen in the alcohol-fed rats but not in control diet group. In in vitro studies, alcohol and LPS exerted a synergistic effect on PSC activation, and pancreata exposed to alcohol were more sensitive to LPS-induced damage. This may be caused by increased sensitivity to necrotic cell death rather than apoptotic cell death (42). Withdrawal of alcohol led to the resolution of pancreatic lesions including increased PSC apoptosis and decreased fibrosis (163).

Smoking is a strong and independent risk factor of pancreatitis. Cigarette smoke dose dependently potentiates the amount of pancreatic injury generated by ethanol alone (63, 179). Pancreatic stellate cells can be activated by clinically relevant concentrations of cigarette smoke components (90).

In a cyclosporin model of alcoholic CP developed by Gukovsky et al. (57), the addition of cyclosporin and caerulein in relevant concentrations of cigarette smoke components (90). Smoking is a strong and independent risk factor of pancreatitis. Cigarette smoke dose dependently potentiates the amount of pancreatic injury generated by ethanol alone (63, 179). Pancreatic stellate cells can be activated by clinically relevant concentrations of cigarette smoke components (90).

In in vitro studies, alcohol and LPS exerted a synergistic effect on PSC activation, and pancreata exposed to alcohol were more sensitive to LPS-induced damage. This may be caused by increased sensitivity to necrotic cell death rather than apoptotic cell death (42). Withdrawal of alcohol led to the resolution of pancreatic lesions including increased PSC apoptosis and decreased fibrosis (163).

Perspectives

There are many models available for both acute and chronic pancreatitis. These models have been discussed in many excellent reviews (4, 43, 68, 78, 91, 143, 184). However, investigators studying pancreatitis face several challenges. The first and most major challenge is the clinical relevance of the models. Unfortunately, many models use non-clinically relevant stimuli, while others fail to reproduce human diseases, even with human-related etiological insults. Thus the mechanisms found from these models should be interpreted with caution. The second is that reproducible rodent models for some forms of human pancreatic inflammatory diseases with known etiologies (e.g., hereditary pancreatitis, cystic fibrosis) have yet to be developed. Last, current research largely focuses on the initiating mechanisms. Effective preventive and therapeutic drugs for the human pancreatitis are still lacking. Clinically relevant models are urgently needed for developing and testing interventions.

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Themes

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