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Chemically Induced Models of Pancreatitis

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I. Acute Pancreatitis

A. Cerulein

Cerulein-induced pancreatitis is one of the best characterized animal models of pancreatitis. First described in 1977, this model is highly reproducible and economical, and therefore widely used to generate both acute and chronic pancreatitis in mice and rats (17, 33).

Cerulein is a cholecystokinin (CCK) analogue derived from the Australian tree frog *Litoria caerulea* which provokes digestive enzyme secretion in the pancreas in both humans and rodents. Cerulein acts on two different CCK receptor subtypes, CCK1 (previously named CCK-A) and CCK2 (previously named CCK-B) (13). Pancreatic acinar cells from various species express CCK1, CCK2, or a combination of these CCK receptor subtypes. In rodents, it appears that two mechanisms are involved, one indirect and one direct. The indirect mechanism involves the binding of CCK to CCK1 receptor expressed in afferent neurons that influence pancreatic secretion via vagal-vagal loop with the final mediator being acetylcholine acting at m3 muscarinic cholinergic receptors. This neurogenic mechanism has been reported in humans (30). The direct mechanism involves binding of CCK to CCK1 receptors on pancreatic acinar cells leading to an increase in intracellular Ca^{2+} and secretion of digestive enzymes.(10) Human pancreatic acinar cells do not respond directly to CCK receptor activation, likely due to insufficient level of CCK receptor expression (11).

Doses of cerulein beyond those that cause the maximum pancreatic secretion of amylase and lipase result in a dysregulation of the production and secretion of digestive enzymes, particularly, the inhibition of pancreatic enzyme secretion and an elevation in their serum levels, cytoplasmic vacuolization and the death of acinar cells, edema formation, and an infiltration of inflammatory cells into the pancreas (10, 34). Cerulein also contributes to oxidative stress by activating NADPH oxidase, which is a major source of reactive oxygen species during inflammation and apoptosis in pancreatic acinar cells (13).

Cerulein can be administered via intraperitoneal (IP), intravenous (IV) or subcutaneous injections (8). The IV route, which allows for continuous cerulein administration (at doses of 5-50 mg/kg/h), is thought to be the best way of administering the

hormone to rats; however, it is not commonly used due to the requirement of central venous cannulation and anesthesia. For this reason, the cerulein-induced model is usually elicited by the IP or subcutaneous injections of several (4-12) hourly doses at 50 μg/kg of the secretagogue. In rats and mice, pancreatic injury is induced within an hour of the start of cerulein administration, and the peak of histological changes (interstitial edema, inflammation, and acinar cell injury/death) is noted 3-6 hours after the start of secretagogue administration. By 24 hours after the start of supramaximal secretagogue stimulation, these changes begin to resolve, and one week later, the pancreas appears to be morphologically normal (22). The severity of the disease can be adjusted by varying the dose and number of cerulein injections. Similar to the clinical characteristics of human acute pancreatitis (AP), the severity of cerulein-induced AP is more pronounced in aged mice, which exhibit higher mortality rates (29).

Advantages of using this model include the fact that it is non-invasive, spares bile duct and endocrine cell injury, allows easily controlled grades of injury, is highly reproducible and applicable in several different species such as mice, rats, hamsters, and dogs. In addition, isolated acini can also be exposed to supramaximally stimulating concentrations of cerulein and, in this way, *in vivo* studies can be complemented by *ex vivo* studies under more completely controllable conditions. This model is particularly suitable for investigating cellular changes observed in the early phases of AP and the autoactivation process of digestive enzymes. Major limitations of this model include the inability to mimic the clinical situation, which may not involve hypersecretion of CCK. In addition, this model, which generates a mild and self-limited pancreatitis phenotype, is limited in the study of more severe or destructive disease which confers clinical morbidity and mortality (12). Overall, cerulein generates edematous AP similar to human edematous pancreatitis. Since the histopathological findings in cerulein-induced AP closely resemble those of AP in humans, this model is considered as a representative model of mild AP and is widely used to study the pathogenesis of AP in terms of intracellular enzymatic activation and mechanisms of inflammatory cell infiltration.

B. L-Arginine

It has been known for the last 60 years that non-physiologic or excessive doses of natural amino acids (AAs), including ethionine, methionine, azaserine, cycloleucine, β-3-thienyl-DL-alanine, β-3-furyl-DL-alanine and, more recently, L-arginine, L-ornithine and L-lysine can cause damage to the exocrine pancreas. Excess dietary or exogenous AAs are believed to cause damage to acini via endoplasmic reticulum stress which disrupts normal protein synthesis. Other proposed mechanisms include mitochondrial injury, oxygen free radicals, nitric oxide, and inflammatory mediators (14, 41).

The L-arginine-induced AP model was first described by Mizunuma *et al*. (25), and Tani *et al*. (36) n rats. A single IP injection of 5 g/kg L-arginine selectively destroyed nearly all the pancreatic acinar cells. It was not until 2007 that Dawra *et al*. (3) characterized the L-arginine-induced model (2×4 g/kg IP in one-hour intervals) in BALB/c and C57BL/6 mice as well. L-arginine induces severe necrotizing AP in a dose- and time-dependent manner, making it an ideal model to study the different phases of pancreatitis (14).

After a single IP injection of 500 mg/100 g body mass of L-arginine, Kishino *et al.* observed pancreatic degeneration started with

disorganization of the rough endoplasmic reticulum. The main changes in acinar cells after 24 hours were partial distension of the endoplasmic reticulum. At this time, large, sequestered areas in the cytoplasm contained disarranged rough endoplasmic reticulum and degraded zymogen granules. Forty-eight hours after L-arginine injection, dissociation and necrosis of acinar cells were noted. Subsequently, the necrotic cells were replaced by interstitial tissue composed of leukocytes and fibroblasts (14). Major histopathological changes were observed within 4 to 12 hours. These pathological manifestations included vacuolized acinar cells as well as a small infiltration of neutrophils and monocytes. Acinar cells were heavily vacuolized from 24 to 48 hours, and necrosis with massive interstitial edema ensued. The histoarchitecture of the pancreas was severely destroyed, with large areas of necrotic tissue after 72 hours (7). Pancreatic acinar cells began to regenerate within 7 days, and pancreatic architecture appeared almost normal after 14 days (36).

Major advantages of the basic amino acid-induced pancreatitis model are that it is inexpensive, non-invasive, and requires only two IP injections. The L-arginine model severity can parallel that of bile salt–induced pancreatitis under specific conditions. However, significant variability in AP severity occurs between mouse strains and between mice and rats. In one study, in FVB/n and C57BL/6 mice, the pancreatic necrosis rate (about 50%) was significantly higher than that observed in BALB/c mice using 2×4 g/kg 10% L–arginine, but euthanasia was necessary in a large proportion of animals. The IP injection of lower L-arginine concentrations (e.g. 5–8%) in case of the 2×4 g/kg dose, or other L-arginine doses (3×3 or 4×2.5 g/kg, 10%) were better for inducing AP. Furthermore, the concentration of the administered L-arginine-HCl solution makes a huge difference in whether the animals survive the treatment. High L-arginine concentrations like 30%--which are well tolerated by rats--actually kill mice. Thus, lower (5–10%) concentrations should be injected even if this means considerably more fluid volume (15). There are also reports in the literature to indicate that even a single dose of 500 mg/100 g IP causes significant mortality in rats while use of a double dose (2x250 mg/100 g at 1 hour interval) reproducibly causes pancreatitis without mortality (26).

C. Choline deficient ethionine-supplemmented (CDE) Diet

In the 1930s it was found that a diet deficient in the essential nutrient choline (a member of the vitamin B complex and component of cell membranes) damaged the pancreas (5). When mice were fed a choline-deficient diet enriched with ethionine, a derivative of methionine (the CDE diet), they developed severe necrotizing AP. The CDE diet includes vitamin free casein (10%), alpha soy protein (10%), DL-ethionine (0.5%), sucrose (56%), lard (20%), and mineral mix (3.5%) (9). This model was standardized by Lombardi *et al; (21)* 4- to 6-week-old female mice weighing 10 to 14 g were food restricted for the first 24 hours, then allowed 3 g of CDE diet per mouse for the next 24 hours while checking for mortalities every 24 hours thereafter.

The CDE diet model produces acute hemorrhagic pancreatitis within about 5 days with a mortality rate reaching nearly 100%. The mechanism remains unclear, but it is likely that ethionine disrupts phospholipid metabolism of membranes that are involved in the intracellular transport and secretion of pancreatic enzymes. A choline-deficient diet would potentiate the activity of ethionine because a choline-deficient diet also induces changes in membrane phospholipids of cellular organelles. Thus,

normal luminal exocytosis is blocked, and zymogen granules accumulate in the cytoplasm. This disruption of stimulus-secretion coupling in acinar cells appears to occur at a step after hormone-receptor binding but before apical Ca^{2+} release. Other proposed mechanisms include impaired hormone-stimulated generation of inositol phosphates, activation of intrapancreatic proteases, and vascular changes (5, 18-20). Although the detailed mechanism for hemorrhagic lesions remains unclear, destruction of the elastic tissue of intrapancreatic vessels may also occur in this model.

Although this model initially was developed using young female mice, male mice can be used in this model after estrogen administration (31). Similar results have been reproduced in rats (32). A relatively consistent mortality rate is highly advantageous for evaluating the efficacy of new drugs being developed to treat pancreatitis. Like other chemical models of AP, the severity of pancreatitis differs among strains, highest in C3H/HeJ and CBA/J, moderate in BALB/c, and mildest in C57BL/6J and JF1 (37). Other disadvantages are that the diet is costly, and its implementation requires significant effort, with on-site protocol standardization and careful monitoring of dietary intake and animal status for each new experiment.

II. Chronic Pancreatitis

A. Cerulein

Recurrent episodes of AP lead to chronic pancreatic injury in humans. Mimicking the human pathogenesis, repeated bouts of cerulein-induced AP over the course of several weeks causes chronic injury to the pancreas with resultant collagen deposits and fibrosis (40).

Typically, chronic pancreatic injury is induced in 8-week-old mice by repeated IP injections of cerulein, 50 μg/kg hourly for six doses. Treatments are given every other day for a total of three treatments per week for four consecutive weeks. This method mimics the atrophy and fibrosis observed in chronic pancreatitis (CP), however there are many variations regarding the amount and frequency of cerulein injections and extent of fibrosis is highly variable (4, 24, 28).

At the beginning of the recovery period, the pancreas produces components of extracellular matrix that temporarily exceed the degradation of the extracellular proteins (2). In addition, profibrotic cytokines are released leading to an environment that favors fibrosis. One of the most potent fibrogenic modulators is TGFβ1, which is overexpressed in pancreatic acinar and stromal cells after cerulein-induced pancreatitis (6, 23). In this phase the organ is extremely vulnerable to repeated episodes of AP and will not be able to degrade the ECM components, which finally promotes fibrosis after a number of repeated injuries (27).

Recently, a new combined model of IP injections of ethanol and cerulein was found to successfully induce CP in mice which mimics the initial phases of CP development in alcoholics. IP injections of ethanol provide higher reproducibility compared to ethanol feeding (1).

B. Dibutyltin dichloride (DBTC)

DBTC was reported to cause pancreatic injury in East German ship builders when it was used as a paint component. Toxic to the hepatobiliary system, DBTC causes CP in rats via a single IV injection (DBTC; 8 mg/kg/body weight). DBTC induces acute pancreatic inflammation within 24 hours, which progresses to chronic inflammation in a week,

and then to a progressive fibrotic lesions over the following 2 months, with chronic and acute inflammation (mediated by T cells and macrophages). Pancreatic levels of TGF-β1, a mediator of pancreatic fibrosis, correspond with collagen expression.

Male outbred rats (LEW-1W, Karlsburg, Germany) weighing 150-170 g are used. Animals are maintained under standard conditions and fed rodent chow and water *ad lib*. DBTC is dissolved in 100% ethanol (two parts) and then mixed with glycerol (three parts). DBTC (8 mg/kg body weight) in a volume of 200 μL solvent is injected into the tail vein (35). Given the progressive nature of disease, animals can be evaluated at different time points to examine different features of CP.

C. L-Arginine

Similar to the necrosis-fibrosis theory of repeated AP attacks leading to chronic pancreatic injury (as demonstrated in the Cerulein model), investigators have also explored the use of repetitive intraperitoneal L-Arginine injections in animal models to generate CP (38). Various protocols have been described, however histological changes have been heterogenous and not necessarily representative of changes typically seen in human CP (16, 38). These protocols typically cause acinar cell atrophy, some fibrosis, inflammation, and varying degrees of fat deposition. One group found that repetitive intraperitoneal injections of near-lethal doses of L-arginine results in the replacement of damaged acini with predominantly fatty tissue (as opposed to the fibrotic replacement seen in humans) (39). Because of the heterogeneity of histological results, further work needs to be done to model L-arginine induced CP.

D. CDE Diet

Mice fed an intermittent CDE diet (3 days of CDE diet alternating with 3 days of normal diet) for a prolonged period (≥24 weeks) develop histologic features of CP.

III. References

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