

Basic Experimental Pancreatitis Models for Beginners

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Abstract

Efforts to find an ideal model for pancreatitis date back to 1960's. Many models are suggested since then. Every model has its own advantages and disadvantages. Some of these models test etiology while others simulate the complications of pancreatitis. An ideal model which by itself demonstrates all aspects of pancreatitis including systemic changes is yet to be described. In this review we tried to gather the basic, easy to construct models.

Keywords: Pancreatitis, Experimental Models, Closed Duodenal Loop, Arginine Induced, Ex Vivo Perfusion Model, Duct Obstruction, Taurocholate Injection, Vascular Induced

1. Introduction

Acute pancreatitis (AP) is inflammation of pancreatic tissue which can present in a wide spectrum ranging from edema of the organ to necrosis and hemorrhage. Acute pancreatitis is a multi-etiology disease with controversial physiopathology. Thus, it has an unpredictable course without a targeted treatment [1] which results in high morbidity and mortality.

In the clinical setting gallstone obstruction is the most common cause (30%-50%) of AP. Alcoholism is the second most common cause and recurrent alcoholic AP leads to chronic pancreatitis. Infection, autoimmune response, trauma, hyperlipidemia, hyperparathyroidism account for nearly 10% of AP cases [2].

There are many experimental studies which try to identify the pathogenesis and treatment options for pancreatitis. In this review we tried to evaluate the differences between models and the particular methodologies of each experimental model with outline of evolution of each technique.

2. Closed Duodenal Loop (CDL) Induced Pancreatitis

In physiological conditions there is a pressure difference between pancreatic duct (PD), sphincter of Oddi and duodenum. This prevents duodenopancreatic reflux. CDL, by violating this normal state, increases intraduodenal luminal pressure causing reflux of duodenal fluid

to PD causing pancreatitis. Closed loop is constructed with duodenum surrounding the opening of the PD.

This model was first described by Seidel and popularized later by Pfeffer [1]. The first species used were dogs but rats were used later on. In dogs, duodenum is resected distal to pylorus and opening of PD then double layer sutured. The continuity of the gastrointestinal tract is maintained by gastrojejunostomy and common bile duct is obliterated with sutures [3].

Mc Cutcheon and Race injected barium sulphate in to the loop and retrieved it inside the PD intraoperatively [4]. The same authors after severing the mucosal valvulae of Oddi found that under physiologic intraduodenal pressures reflux into PD occurred [5]. In this model if PD is sutured pancreas atrophies. Byrne and Joison's modification enables easy suturing of distended PD after injection of secretin [6].

Contributions to this method were made by Chetty *et al.* via either filling the duodenum with *Proteus* and *E coli* infected human bile [2] or filling duodenum with auto-claved human bile [7].

This model also explains the hyperamylasemia and duodenopancreatic reflux in afferent loop obstruction after Billroth 2 gastrectomy [8].

The histopathological changes in pancreas after this method are studied in detail by Rao *et al.*

Mild to moderate pancreatitis is seen in six hours and hemorrhagic pancreatitis becomes widespread after 18 hours. Although changes similar to human pancreatitis is encountered, this could also be the result of systemic

response to duodenal surgery [9].

Dickson *et al.* assessed the applicability of CDL on humans [7]. Their criticism was as follows:

- Generally mild pancreatitis occurs
- Infected duodenal fluid flows into PD and causes bacterial infiltration of pancreas while this is not the usual case in human pancreatitis
- Transmural duodenal necrosis and cholangitis kill the animal
- Peritoneal sepsis and bacteremia accompanies the pancreatitis at all times in CDL whereas

This is not a common finding in human pancreatitis [10].

3. Diet Induced Pancreatitis

The relationship between feeding with ethionine and acute pancreatitis is well known [11].

Ethionine is toxic to pancreatic acinar cells [12]. It inhibits phospholipid metabolism intracellularly [13,14]. Lombardi *et al* induced acute hemorrhagic pancreatitis in female mice with 0.5% ethionine enriched diet [15]. Widespread intra-abdominal fatty necrosis follows pancreatitis. If feeding is limited to 24 hours mortality is 55-60%. If fed ad libidum this diet is 100% lethal in 5 days [16]. Histopathologic and gross examination of pancreas between 48-72 hours after 24 hours feed did not show any pancreatic damage [17]. When animals are fed with choline, pancreatitis does not develop [18]. Choline takes up the ethyl groups liberated during breakdown of ethionine. Female sex steroids seem to promote development of pancreatitis so either young female mice or oestrogen treated male mice are preferred [19].

Diet without choline exerts synergistic effect to ethionine causing intrapancreatic activation of zymogens leading to massive hemorrhagic necrosis. The subcellular mechanism underlying this is the inhibition of membrane lipid synthesis resulting in breakdown of endoplasmic reticulum and release of autophagic vacuoles. The end result is autolysis [20-22].

The diet model appears to be a good approximation of severe necrotizing human pancreatitis. Both the gross and histological appearance of the pancreatic and peripancreatic inflammation as well as the clinical and biochemical course of diet-induced pancreatitis resemble human disease. Ascites, acidosis, hypoxia and hypovolemia occur in this model like in human pancreatitis. The time course of the morphological and biochemical alterations have extensively been studied and are thus well defined in this model. However, small size of the animals used is a limitation for evaluation of surgical procedures and new diagnostic tools [23].

4. Arginine Induced Pancreatitis

Apart from ethionine, other amino acids like arginine can also induce pancreatitis. High dose intraperitoneal injection of 500 mg/100 gr arginine can cause acute necrotizing pancreatitis in rats, rabbits and mice [24-28].

The possible mechanisms underlying the effect of arginine is via excessive nitric oxide production, lipid peroxidation and inhibition of protein synthesis [29-31].

Dose and exposure of arginine determines the severity of pancreatitis in this model. The changes range between interstitial edema, inflammatory infiltration, acinar degeneration to massive necrosis after 250 mg/kg and 450 mg/100 kg of injections respectively [32,33].

In addition to ease in controlling the destruction, arginine exerts minimal effect on other tissues which makes this model a plausible non invasive method for experimental pancreatitis [34].

The only drawback is its weak clinical relevance which made this method get replaced by other models.

5. Secretagogue Induced Pancreatitis

Cerulein is a decapeptide analogue of cholecystokinin (CCK) derived from the skin of the amphibian *Hydra caerulea*. When given either 1-5 ng/kg intravenous (iv) bolus or 0.25-1 ng/kg/min iv infusion or 50-100 ng/kg subcutaneously this substance increases pancreatic secretions [22]. If administered in supramaximal doses it causes edematous pancreatitis by increasing pancreatic protein secretions [35].

Cerulein interferes with packaging of zymogens and lysosomal hydrolases after synthesis in endoplasmic reticulum leading to intracellular activation of trypsinogen [36]. In 48 hours after infusion zymogen granules start fusion with lysosomes resulting in inflammation and acute pancreatitis [37,38].

The usual way of administration is by a catheter inserted in internal jugular vein of the rat at a rate of 1-2 ml/hour [39,40]. Cerulein can be diluted in normal saline and infused iv in 3-5 hours [41,42].

Cerulein can also be administered intraperitoneally [43-45]. Multiple injections can be done in one hour intervals with 5-200 µg/kg doses.

Subcutaneous delivery can be achieved in multiple injections with 25-50 µg/kg dose [46,47].

In order to increase the degree of pancreatitis more than one model can be used. Schmidt *et al.* combined iv cerulein (5 µg/kg/hr) with low pressure intraductal glycodeoxycholic acid infusion [48]. They observed that edema, acinar necrosis, inflammation and hemorrhage were profound.

Schoenberg *et al.* infused cerulein (5 micrograms/kg per hour) for 30 minutes, 3.5 hours, and 12 hours in rats.

No damage was seen after 30 minutes whereas after 3.5 hours interstitial edema, intravascular migration of granulocytes, zymogen degranulation and acinar cell necrosis was seen. After 12 hours, histological evaluation showed pronounced zymogen degranulation, extensive tissue necrosis, and migration of granulocytes into the tissue. Amylase and lipase activities increased 15 and 35-fold respectively during this time [49].

6. Duct Obstruction Induced Pancreatitis

This model mimics benign and malignant partial or complete obstruction of PD. The model reflects tumors, gallstone disease, trauma in the clinical setting. The surgical manipulation is simple, requiring either ligation of the common biliopancreatic duct or obstruction of the pancreatic duct by vertical cannulation or insertion of a balloon-tipped catheter. The point of obstruction is close to the entry to duodenum, much like gallstone obstruction at the ampulla of Vater [50,51].

Duct obstruction leads to acinar atrophy without causing pancreatitis. The physiological mechanism of this model is thought to be similar to that of the CDL technique. It is postulated that bile reflux by triggering intrapancreatic digestive enzyme activation accounts for the major pathological factor in this model. Duct obstruction induced pancreatitis can be complicated with other stimulations and surgical manipulations.

For example, caerulein or secretin can be administered to the animal together with duct ligation to exaggerate the pancreatic secretions [52-54].

The severity of pancreatitis produced by the duct obstruction model varies depending on the animal species used for experiment. In dogs physiological pressure in PD is 30 cmH₂O [55]. When PD is ligated pressure rises to 40-80 cmH₂O in 6-12 hours. Fluid accumulation in PD starts in 10-30 hours and continues up to 40 hours [56, 57].

After 24 hours the equilibrium between secretion and PD obstruction is maintained which stops further PD pressure rise and parenchymal water content [55,56]. One week after PD ligation acinar cell zymogen content was found to be decreased, rough endoplasmic reticulum is fragmented, golgi apparatus function is lost, autophagic vacuoles appear and exocrine pancreas is replaced with fibrous tissue [57]. These changes occur faster in rats than dogs. In rats, main bile duct passes through pancreas and many small pancreatic ducts join with it. In order to prevent flow of only pancreatic secretions, duodenum is separated from transverse colon and a polyethylene tube inserted in to the proximal part of main bile duct [58].

In rabbits PD obstruction does not lead to the morphological changes of pancreatic trauma or inflammation but exocrine pancreas atrophies. PD is directly cannu-

lated and located in vertical direction in rabbits. This is thought to mimic obstructive biliary pancreatitis in humans [59]. This technique had been also utilized for assessment of effect of pancreatic enzymes on small intestine brush border enzyme activity [60].

Opposums were also used for this model. Opposum's bile structure closely resembles human biliary system. Bile tract has a single terminal end combining with PD 2-3 cm before opening into duodenum.

Occlusion of the common biliopancreatic duct causes acute hemorrhagic pancreatitis and results in 100% mortality in 14 days [61].

When it is sutured adjacent to duodenum, pancreatic edema forms in 6 hours and peaks in 12 hours. At this stage fatty necrosis and parenchymal hemorrhage start to appear and infiltration by inflammatory cells occurs [61-63].

Another variant of this technique is partial obstruction of the pancreatic duct studied on cats. After exposure of the PD it is partially sutured proximally. PD is cannulated from the tail and secretions are collected. The secretion is increased with secretin and CCK. It was found that changes in pancreas depend on the degree of obstruction. If it exceeds 75%, acinar atrophy and decrease in response to secretin and CCK stimulation occurs.

Lesser degrees of obstruction only impairs enzymatic secretions. Three months after recovery from obstruction neither enzyme nor bicarbonate secretions return to normal and tissue regeneration does not occur [64]. The disadvantage of this technique is difficulty in determining the degree of obstruction which is assessed by instilling vinyl chloride in retrograde fashion in to PD and examining it under microscope.

The duct obstruction model has high clinical relevance in that it simulates obstruction induced AP. Moreover, this induction method is quick and does not require sophisticated surgical techniques. These advantages have made this model a favorite for investigating the pathophysiology, as well as the therapeutic treatment of obstruction induced pancreatitis. It does not require administration of systemically active substances. Although not used as frequently as it had been, it should be kept in mind when experimenting chronic obstructive pancreatitis as acinar cell loss and fibrosis is encountered in long term ductal obstruction.

7. Ex Vivo Perfusion Model

This model was first described by Saharia *et al.* in 1977. It enables experimentation of different etiologies of pancreatitis [65].

The technical details are as follows: Pancreas of the dog is mobilized with the duodenal segment adjacent to it. Splenic artery and superior mesenteric artery distal to

inferior pancreaticoduodenal artery is cannulated. Portal vein is cannulated and incoming venous blood is collected in a reservoir. A 16 G polyethylene catheter is placed in PD via a small duodenotomy. The circulation in this model is first started with 200ml of autologous blood. Human serum albumin (2.5 g), glucose (500 mg) and sodium bicarbonate (20 ml) is added to the perfused blood. During the experiment pH should be kept at 7.40. Blood glucose level is fixed at 100 mg/dl [65,66].

After the pancreas is harvested it is stored on a plexi-glass surface in a humid environment. The blood in venous reservoir is passed through 95% oxygen and 5% carbon dioxide supplying oxygenator. Another pump pushes back the blood via the splenic and superior mesenteric arteries. The index for blood flow is either 20-30 ml/min or 1 ml/min/6 gr of tissue [67]. Temperature of the perfusate is adjusted to 37°C. Partial obstruction can be added to the model by inserting 25 G catheter in to pancreas [68]. Increasing the blood flow results in edema [65]. Albumin added previously decreases edema and hemorrhage of the pancreas [69].

Alcoholic pancreatitis can be induced by adding free fatty acids or acetaldehyde in to perfusate [65,70-73]. Different etiologies can be studied by changing the flow rate, oxygen content, delaying perfusion or adding cerulein [71,74,75].

Although expensive, ex vivo perfusion model is a plausible model as the organ is isolated from body preventing systemic factors intervening. A complex equipment which has a propensity for breakdown in about 4 hours made this model remain unpopular.

8. Duct Infusion Pancreatitis

Cannulation of the pancreatic duct provides another way of inducing an experimental AP model. Once the cannula has been implanted, an exogenous substance can be infused into the pancreas via the pancreatic ductal system. Several substances have been used as inducers of pancreatitis in this method. These have included stimulating factors and toxic substances such as bile acids (taurocholate or glycodeoxycholic acid), ethyl alcohol, peracetate and tert-butyl hydroperoxide. The most common of all these substances are bile acids [76-78].

In rats biliopancreatic duct is catheterized with 24 G polyethylene tube and ligated [79]. Main hepatic duct is clamped under liver and intraductal infusion is started. Glycodeoxycholic acid prepared in glycylglycine buffer (pH = 8) is recommended as infusate. Infusion should be in 1.5 minutes, with 30 mmHg pressure and 0.1-0.5 ml volume.

In dogs trypsin is used along with bile acids [80]. After duodenectomy accessory pancreatic duct is cannulated with 1.5 mm tube and bile acids and trypsin in 1:1 ratio,

0.5 ml/kg solution is given with 140-150 mmHg pressure. Infusion of bile acids is a fast and cheap way of inducing pancreatitis resembling human pancreatitis. Mortality can be controlled with changing the quantity of the infusate. Both edematous and hemorrhagic pancreatitis can be induced. Major disadvantage is different response of different species to infusion.

Taurodeoxycholate (0.2 ml, 0.025 molar, glycylglycine-NaOH, pH: 8.0) is the second most used substance. In rats it is delivered 0.04 ml/min with an infusion pump [81]. Schoenberg *et al.* recommend that the pressure does not exceed 15 cmH₂O during infusion [82]. In 3.5 hours 20% of rats die while in 57% die in 12 hours. After 3.5 hours fulminant hemorrhagic pancreatitis is seen under light microscope. Zymogen degranulation, 50% cell necrosis, mild tissue edema, inflammatory cell infiltration is observed. In 12 hours almost all of the acinar cells undergo necrosis.

In dogs 20 G catheter is placed in pancreatic duct and 1.8 gr sodium taurocholate with 250.000 U benzoyl L-arginine ethyl ester hydrochloride crystal trypsin in 20 ml sorenson buffer infusion is carried on for 30 minutes not exceeding a pressure of 30 cmH₂O [83]. Intraductal taurocholate infusion causes severe pancreatitis. Inflammation is not homogenous and mostly on the head of pancreas [84].

The repeatability and clinical relevance associated with the duct perfusion induced pancreatitis make it an excellent experimental model for pancreatitis studies. However it requires careful monitoring of perfusion pressure and an invasive surgery. Intraductal infusion of saline alone has been reported to induce mild pancreatitis [85]. Findings demonstrated that pancreatic injury is attributable not only to the exogenous substance infused but the combination of the exogenous substance and the hydrostatic pressure associated with the infusion. The presence of exaggerated hydrostatic pressure makes it less clinically relevant relative to other models like the duct obstruction model. Despite this drawback, the duct perfusion model is the most commonly used pancreatitis model because of its similarity to clinical pancreatitis [86,87].

The confounding effects of increased ductal pressure can be ameliorated by antegrade perfusion model which is first studied by Reber *et al.* [88]. They used cats for this purpose. The technique requires removal of spleen and greater omentum. Two catheters are placed in PD; one through duodenum and the other from the tail of pancreas [89]. Perfusion is maintained by a pump at a rate of 0.2-3.75 ml/hour for two hours. At this rate intraductal pressure does not exceed 20 cmH₂O. Reber *et al.* recommend use of certain substances (bile, aspirin, hydrochloric acid, ethanol, secondary bile acids) to overcome pancreatic canal barrier and increase permeability.

9. Vascular Induced Pancreatitis

Acute pancreatitis is encountered after cardiopulmonary bypass [90]. Changes in vascular perfusion of pancreas leads to pancreatitis in many animals including dogs, rats, cats [91,92].

Vascular perfusion can be changed by altering either of inflow, outflow or microcirculation of the organ.

In 1962 Pfeffer *et al.* used 8-20 μ gr polyethylene microspheres to occlude superior pancreaticoduodenal arteries. With this technique irreversible occlusion of terminal arterioles is achieved impeding microcirculation [93] causing hemorrhagic pancreatitis in 11 hours. Using larger particles only result in pancreatic edema.

Permanent occlusion of the superior pancreaticoduodenal artery results in elevated serum pancreatic enzymes and necrosis. However, the artery occlusion induced pancreatitis model has weak clinical correlation because pancreatitis induced by artery occlusion in humans is rare [94].

Pancreatic blood flow can also be severed by occlusion of pancreatic veins, either by ligation or injection of microspheres. Splenic or gastroduodenal vein occlusion has been shown to lead to elevated serum amylase and histopathological findings [95].

One of the methods for suppressing the inflow to the pancreas is to create a low flow state by inducing hypovolemic shock. In 1987, Brasilia *et al.* showed that withdrawal of 30% to 35% of blood from dogs created hypovolemic shock. After 3 hours of hypovolemia canine pancreases showed a significant weight gain.

Microscopic analysis revealed significant edema, hemorrhage, acinar cell necrosis, and fat necrosis. Hypovolemic shock induced pancreatitis imitates the pancreatitis observed after extensive surgery in the clinical

setting [96].

The major disadvantage of vascular induced pancreatitis is the effects of intense surgical trauma exerted on the animals. It necessitates extensive bleeding, complex surgical protocol and continuous analgesia. It is usually applied to large animals like dogs and pigs. In hypovolemic shock induced pancreatitis, the damage is not localized to pancreas but systemic. Venous occlusion and disturbance of pancreatic microcirculation have low repeatability. Thus, the vascular-induced pancreatitis model has become less popular [97].

10. Intraparenchymal Taurocholate Injection

This model is worth mentioning because it is highly reproducible any easy to apply.

When injected into tail or body of rat pancreas with 25 G needle, 1 ml 10% solution of taurocholate causes necrotic lobules, fatty necrosis in and around pancreas [98].

Paran *et al.* showed that six hours after injection plasma activities of amylase, lipase and lactate dehydrogenase increase and twenty four hours after injection pancreatic morphological changes with good correlation to clinical findings and mortality were seen [99].

11. Conclusions

Endeavors to identify an ideal model for pancreatitis date back to 1960's. The models tested are summarized in **Table 1**. Each model has its own advantages and drawbacks. The researcher should choose among the models depending on what he wants to test in his experiment, the infrastructure of his laboratory and his surgical skills.

Table 1. Summary of the models reviewed.

| | Animals | Advantages | Disadvantages |
|--------------------------------------|---------------------------------|--|--|
| CDL | Rats, dogs | Clinical relevance | Complex surgical technique High surgical trauma Not suitable for small animals |
| Diet Induced | Mice | Non invasive, reproducible | Weak clinical relevance Suitable for small animals |
| Arginine Induced | Rats, mice, rabbits | Non invasive, easy to control damage, toxicity limited to pancreas | Weak clinical relevance |
| Secretagogue Induced | Mice, rats, rabbits, dogs, pigs | Non invasive, easy to control damage Reproducible | Weak clinical relevance |
| Duct Obstruction | Rats, rabbits, opossums | Mimics gallstone-obstruction induced pancreatitis | Severe AP only in opossums |
| Ex vivo Perfusion Model | Dogs, pigs | Organ isolated from systemic effectors | Expensive, complex surgery Complex equipment |
| Duct Infusion | Rats, rabbits, dogs, pigs | Clinical relevance, wide spectrum of substances to test | Hydrostatic pressure as confounding factor |
| Vascular Induced | Rats, cats, dogs, pigs | Assesment of operative and venous thrombosis pancreatitis | Complex surgical technique High surgical trauma, expensive Low reproducibility |
| Intraparenchymal Taurocholate | Rats | Highly reproducible, easy to apply | Localized pancreatitis |

Some models test etiology while others simulate the complications of pancreatitis. Combination of models can also be used if single model does not fulfill the needs. Ideal model which by itself demonstrates all aspects of pancreatitis including systemic changes is yet to be described.

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