REVIEW ARTICLE

Review of experimental animal models of acute pancreatitis

KIM HUE SU, CHRISTINE CUTHBERTSON & CHRISTOPHER CHRISTOPHI

Department of Surgery, University of Melbourne, Austin Hospital, Melbourne, Victoria, Australia

Abstract
The underlying mechanisms involved in the pathogenesis of acute pancreatitis are ill understood. The mortality rate of this disease has not significantly improved over the past few decades. Current treatment options are limited, and predominantly aimed at supportive therapy. A key feature of severe acute pancreatitis is the presence of extensive tissue necrosis with both local and systemic manifestations of inflammatory response syndromes. A better understanding of the underlying pathophysiology of severe acute pancreatitis may lead to more targeted therapeutic options, potentially leading to improved survival. Animal models of acute pancreatitis are therefore an essential investigative tool for these aims to be achieved. This review discusses the suitability of recent non-invasive models of acute pancreatitis such as hormone-induced, alcohol-induced, immune-mediated, diet-induced, gene knockout and L-arginine; and invasive models including closed duodenal loop, antegrade pancreatic duct perfusion, biliopancreatic duct injection, combination of secretory hyperstimulation with minimal intraductal bile acid exposure, vascular-induced, ischaemia/reperfusion and duct ligation.

Key Words: Acute pancreatitis, animal models

Introduction
Acute pancreatitis has a relative frequency ranging from 5 to 80 cases per 100 000 population in the Western world. There are several aetiopathological factors, the majority being gallstones- or alcohol-related [1,2]. The severity of clinical presentation varies from a mild, self-limiting form to severe disease complicated by sepsis and multi-organ failure. About 25% of patients with acute pancreatitis have severe disease, with a mortality rate approaching 30–40% [3]. The precise underlying mechanisms of severe acute pancreatitis are unknown. The main features of this condition are pancreatic necrosis and associated sepsis, with both localized and systemic inflammatory response syndromes [4,5]. Specific therapy aimed at the underlying pathophysiological pathways is largely experimental and so far ineffectual in human studies.

The pathophysiology event of experimental acute pancreatitis consists of the activation of pancreatic enzymes within acinar cells, the release of these activated enzymes in the interstitium, the autodigestion of the pancreas, and the release of activated pancreatic enzymes and other factors into the circulation that result in the development of multiple organ dysfunction [6–9]. Currently, the factors that determine the ultimate severity of the acute pancreatitis remain uncertain [10–15].

Several concepts of the pathophysiology of acute experimental pancreatitis have emerged (see references/review in Frossard [16]). One concept of the disease includes maintaining the stability between anti- and pro-inflammatory cytokines/chemokines [17,18]. It has been shown that T lymphocytes, particularly, CD4+/CD8+ T cells, are important in the development of tissue injury during acute pancreatitis in mice [19]. Another concept of the pathophysiology of acute pancreatitis includes the fine balance between necrosis and apoptosis. It has been hypothesized that apoptosis might be a favourable response to acinar cell injury, suggesting that induction of apoptosis can effectively reduce the severity of acute pancreatitis [20–22] and may be beneficial in the clinical management of acute pancreatitis. An additional concept includes having a favourable ratio between reactive oxygen species and free radical scavengers. Animal models have been shown to exhibit rapid increases in the concentration of peroxidation products both in pancreatic tissue and serum, with peak changes...
during the first 6 h after acute pancreatitis induction [23–27]. It has been suggested that reactive oxygen species play a pivotal role in signal transduction [28], and may cause alterations in cytoskeleton function [29]. However, results of well-designed clinical studies have yet to prove the benefits of enzyme scavengers to individuals suffering from acute pancreatitis [30].

Experimental models of acute pancreatitis resembling the human situation are an integral tool to increase the understanding of the complex mechanisms and in devising therapeutic strategies for this disease. Several experimental models of severe acute pancreatitis have been produced. These models may be arbitrarily divided into invasive and non-invasive varieties according to the method of induction of acute pancreatitis.

An ideal model of acute pancreatitis should encompass several features. It should be easily reproducible, with the ability to vary the severity of acute pancreatitis in a standardized manner according to the experimental aims. The morphology and pathophysiology should resemble that of the human situation. This article assesses current models of acute pancreatitis with particular emphasis on their suitability as a research tool for the human disease.

Non-invasive models of acute pancreatitis

Several non-invasive models of experimental acute pancreatitis have been developed in an attempt to investigate the initiation, progression and treatment of this disease (Table I).

Hormone-induced model

Caerulein, a cholecystokinin-pancreozymin analogue, has been used to successfully cause acute pancreatitis in rats [31,32], mice [33], dogs [34], and Syrian hamsters [35]. Acute pancreatitis may be induced by intravenous, subcutaneous or intraperitoneal injection routes. Using this method, proteolytic enzyme secretion may be increased to levels that cause pancreatic acinar autolysis [36]. The intravenous route is the preferred method. It allows accurate control of the infusion rate, and thus control of the timing and severity of the acute pancreatitis [31,34,37]. One hour after infusion of caerulein, progressive interstitial oedema develops and reaches a maximum after 12 h. The dose of caerulein may be gradually increased. Pancreatic enzymes in the blood circulation, pancreatic inflammation, acinar cell necrosis and fat necrosis can be observed following a two-fold increase in the caerulein dose [31,38–41].

Acute caerulein pancreatitis has been shown to be particularly effective for investigating the pathogenesis of pancreatitis-related pulmonary pathology [42,43]. The appearance of the pulmonary injury in rats using this model resembles the early stages of the adult respiratory distress syndrome in humans. The structural changes of acinar cells observed in human acute pancreatitis exhibit many similar characteristics to caerulein-induced acute pancreatitis in rats [44–46]. In particular, specific changes to intracellular membrane systems of acinar cells were similar in both human and caerulein-induced acute pancreatitis. This model appears to simulate the pathophysiology of acute pancreatitis induced by Trinidadian scorpion toxin [47] or anti-cholinesterase insecticide [48] poisoning in humans. Both these agents have been employed to induce experimental acute pancreatitis in dogs [49,50].

The advantages of the hormone-induced model are that it is relatively simple and inexpensive to perform. This model has been frequently used to study the cell biology and pathophysiological events in acute pancreatitis. It is also useful for studying systemic disease manifestation. Apart from investigation of the pulmonary aspects of acute pancreatitis, it has also proved particularly useful in elucidating information on gut endocrine interactions such as secretin and cholecystokinin (CCK) levels. Another advantage of this model is that it allows investigation of healing and regeneration of damaged tissue after the toxic substance has been discontinued.

This model has two major disadvantages. Despite infusion of maximum doses of caerulein, only mild acute pancreatitis develops, with negligible mortality. In addition, the course and severity of the underlying acute pancreatitis are highly variable and thus relatively unsuitable for controlled studies.

Alcohol-induced model

The acute effects of ethanol on the pancreas have been investigated in several animal studies in order to investigate the underlying pathophysiological mechanisms leading to alcohol-induced acute pancreatitis. Animal models of acute pancreatitis, induced by acute ethanol application alone, have been difficult to produce [51,52] and require prior sensitization with other agents [53–56] to allow significant pancreatic damage to occur. Developing an experimental model requiring direct ethanol installation via the pancreatic duct is not relevant in a clinical situation. This is because installation of any agent corresponding to concentration and pressure would result in pancreatic injury. Thus, acute ethanol administration has been combined with other substances to study the pathophysiological events. These models have proved particularly useful to study specific alcohol-induced effects such as changes to pancreatic blood flow and the microcirculation, the effect on pancreatic acinar damage by alcohol-related free oxygen radical generation, metabolites and the effect on pancreatic regeneration (see references/review in Schneider et al. [57]).
<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Clinical relevance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone-induced</td>
<td>- Causes acute pancreatitis in a variety of animals, i.e. rats, mice, dogs and Syrian hamsters</td>
<td>- Only mild acute pancreatitis develops</td>
<td>- Pulmonary injury in rats resembles the early stages of the adult respiratory distress syndrome in human</td>
<td>30–34</td>
</tr>
<tr>
<td></td>
<td>- Can induce acute pancreatitis by a number of injection routes, i.e. intravenous, subcutaneous or intraperitoneal; the preferred method is intravenous route</td>
<td>- Negligible mortality</td>
<td>- Structural changes of acinar cells are similar to human acute pancreatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Allows accurate control of the infusion rate, thereby enabling control of the timing and severity of acute pancreatitis</td>
<td>- High variability in the course and severity of underlying acute pancreatitis and thus unsuitable for controlled studies</td>
<td>- Specific changes to intracellular membrane systems of acinar cells resemble human acute pancreatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Useful for studying cell biology, gut endocrine interactions such as secretin and CCK levels, pathogenesis of acute pancreatitis-related pulmonary pathology, systemic disease manifestation, and healing and regeneration of damaged tissue after the toxic substance has been discontinued</td>
<td>- Inexpensive to perform</td>
<td>- Simulates acute pancreatitis induced by Trinidadian scorpion toxin or anti-cholinesterase insecticide poisoning in humans</td>
<td></td>
</tr>
<tr>
<td>Alcoholic-induced</td>
<td>- Useful for studying changes to pancreatic blood flow and the microcirculation, the effect on pancreatic acinar damage by alcohol-related free oxygen radical generation, metabolites and the effect on pancreatic regeneration</td>
<td>- Animal models of pancreatitis, induced by acute ethanol application alone, have been difficult to produce significant pancreatic damage, and thus require prior sensitization with other agents</td>
<td>- Lack of correlation with the clinical situation</td>
<td>50, 53, 56, 57, 60–66</td>
</tr>
<tr>
<td></td>
<td>- Has been used in several animal models, i.e. rats, cats and dogs</td>
<td>- Lack of reproducibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Various route of ethanol administration, i.e. intravenous, oral and direct intragastric instillation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Relatively simple</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Cheap to perform</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Selectively lessens pancreatic blood flow and microcirculation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Gene knockout animals may be used to determine the effect of genetic factors on the development of acute alcohol-related pancreatic injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune-mediated</td>
<td>- Possible application in the field of drug- or toxin-induced acute pancreatitis</td>
<td>- Challenging to set up in laboratory</td>
<td>- Clinical relevance uncertain</td>
<td>72, 73, 76–79</td>
</tr>
<tr>
<td>Model</td>
<td>Advantages</td>
<td>Disadvantages</td>
<td>Clinical relevance</td>
<td>References</td>
</tr>
<tr>
<td>------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Diet-induced</td>
<td>• Simplest method to study acute haemorrhagic pancreatitis</td>
<td>• Species-specific; may only be used in mice, whose small size causes technical difficulties</td>
<td>• Produces severe necrotizing that approximates human pancreatitis</td>
<td>82, 84</td>
</tr>
<tr>
<td></td>
<td>• Well-established</td>
<td>• Sex-specific; female mice</td>
<td>• Gross and histological appearance of the pancreatic and peripancreatic inflammation, and clinical and biochemical course of diet-induced pancreatitis, resemble the human disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Cheap</td>
<td>• Variable onset of acute pancreatitis</td>
<td>• Ascites, acidosis, hypoxia and hypovolaemia similar to human acute pancreatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• High reproducibility</td>
<td>• Requires careful monitoring to ensure that intake of the CDE diet is the same in different experimental groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No surgical procedure involved</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mortality rate can be controlled at any desired level between 0% and 100% by modifying feeding protocol</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Useful for studying the pathophysiology for acute pancreas and potential experimental treatment by measuring survival, biochemistry, histology, changes in haematocrit, pH and blood gases</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Produces haemorrhage and necrosis with a lethal course</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Inflammatory lesions are homogeneously distributed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene knockout</td>
<td>• Useful for studying the function or effect of a specific gene of interest</td>
<td>• Time-consuming</td>
<td>• Extrapolation of experimental data to humans is difficult</td>
<td>90–99, 101–107</td>
</tr>
<tr>
<td></td>
<td>• Avoids the use of pharmacological manipulations that often cause side effects</td>
<td>• Expensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>Advantages</td>
<td>Disadvantages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-arginine (Arg)</td>
<td>• High reproducibility</td>
<td>• Altering the specific gene from the time of its conception could mean that other protein expressions may result to compensate for the mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mutation may stimulate unforeseen phenotypic changes if a gene is expressed in different tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Expression of two genes may overlap and the alteration in a single gene might mask an abnormal phenotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Long-term administration of Arg produces chronic pancreatitis induction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ability to achieve selective dose-dependent pancreatic acinar cell necrosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Suitable for investigating the early and late phases of acute pancreatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Useful for investigating the insulo-acinar axis, extrapancreatic organ damage and its mechanisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• In a clinical situation, the circulatory, pulmonary, renal and hepatic failure (multi-organ failure) significantly affects the morbidity and mortality of acute pancreatitis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References: 189, 199, 200–203
Several animal models have been used including rats, cats and dogs. The routes of ethanol administration include intravenous, oral and direct intragastric instillation. The morphology of the acute pancreatitis varies according to the animal model, the dose of ethanol administration and the presensitization agents used. It has recently been established that ethanol may lead to the onset of acute pancreatitis via its effect on intrapancreatic digestive enzyme activation, by either directly sensitizing acinar cells to pathologic stimuli [58] or stimulating the release of a secretagogue, CCK, from duodenal I cells [59]. Although the exact role of the various digestive enzymes in initiating pancreatitis remains controversial, it has been established that ethanol can directly interfere with the processes that are involved in digestivezymogen activation (see references/review in Lerch et al. [60]).

The model by Siech et al. [51] combines oral ethanol ingestion and the use of low doses of CCK and secretin to simultaneously stimulate the glands of female Sprague-Dawley rats. Histological changes include evidence of pancreatic duct obstruction, interlobular oedema, cellular inflammatory infiltrates and perilobular fat and parenchymal necrosis [51]. Letko et al. [54] used the same model to test the various modes of ethanol administration and found that it did not influence the development of acute pancreatitis. A number of other studies have found that ethanol can sensitize acinar cells to CCK-induced procarboxypeptidase A1 processing in vitro and can sensitize to various forms of acute pancreatitis in vivo [61–64]. In this way, ethanol is thought to increase stimulation-dependent induction of acute pancreatitis, and thus may be important in ethanol toxicity in the pancreas.

In a study by Wedgwood et al. [65], a cat model of acute pancreatitis was used to study the direct effects of oral ethanol (ethanol 20% v/v in milk, 10 ml/kg every 8 h for 48 h) on the permeability of the pancreatic duct. After the duct was cannulated and perfused with activated enzyme solution, the ethanol-fed cats were induced with acute oedematous acute pancreatitis and the control cats remained unchanged. In a separate study, Friedman et al. [66] studied the effect of ethanol application on pancreatic blood flow in dogs. They found a reduction in pancreatic blood flow in the dogs after intravenous infusion of an ethanol dose that would equate to mild to moderate alcohol intoxication in human. These pancreatic blood flow alterations were not detected at comparable blood ethanol concentrations in pentobarbital-anaesthetized dogs [66].

In another new study, Lu et al. [67] found that trypsinogen and chymotrypsinogen showed distinct patterns of activation in response to supraphysiological concentrations of the CCK analogue caerulein. They also noted that ethanol and other alcohols sensitized the acinar cell to caerulein-induced trypsin and chymotrypsinogen activation, and that other short-chain n-aliphatic alcohols (methanol, propanol, and butanol) advanced the effects of caerulein on the acinar cell. However, when only ethanol was used, neither acute pancreatitis nor zymogen activation was induced in experimental models of acute pancreatitis. Thus, these studies indicate that CCK receptor activation can stimulate various patterns of zymogen activation in pancreatic acinar cells and the extent of activation can be increased by a distinct set of short-chain alcohols. It is still not known, however, whether ethanol and other alcohols mediate these effects by interfering with acinar cell signalling pathways or by affecting acinar cell membrane fluidity.

In 1995, Weber et al. [68] investigated the activity of oxygen radical generating xanthine oxidase in the pancreas of rats treated with either dibutyltin dichloride/ethanol (6 mg/kg/13.7 mg/kg, i.v.), ethanol alone (13.7 mmol/kg, i.v.), or isotonic saline as control. The authors measured the activities of oxygen radical scavengers, superoxide dismutase and glutathione peroxidase, and determined the levels of the lipid peroxidation marker, malondialdehyde. Weber et al. [68] found that tissue imbalance between oxidants and antioxidants may be important in the pathogenesis of dibutyltin dichloride/ethanol-induced acute interstitial pancreatitis, and that although ethanol increases oxygen radical generation, more damage is needed for acute pancreatitis to develop.

Werner et al. [69] studied the acute effects of ethanol on the pancreas by the infusion of non-oxidative products of ethanol metabolism such as fatty acid ethyl esters in Sprague-Dawley rats. They observed increases in pancreatic oedema formation, pancreatic trypsinogen activation and vacuolization of acinar cells. The authors also concluded that fatty acid ethyl esters at concentrations found in human plasma produce an acute pancreatitis-like injury in rats, providing direct evidence that fatty acid ethyl esters can produce organ-specific toxicity. Fatty acid ethyl esters may be important in acute alcohol-induced damage to the pancreas.

Existing alcohol-induced models are relatively simple and cheap to perform. Acute ethanol administration selectively lessens pancreatic blood flow and microcirculation, suggesting that the effect of alcohol might increase ischaemia damage during the evolution of acute pancreatitis with or without underlying chronic disease. Another advantage of this model is that it allows alcohol to directly damage the pancreas by the influence of toxic ethanol metabolites, and perhaps by limitations of pancreatic regeneration. Reproducibility, however, has not been successfully achieved. Furthermore, there is a lack of correlation with the clinical setting. Gene knockout animals may be used to determine the influence of genetic factors on the development of acute alcohol-related pancreatic injury.
Gene knockout model

Gene knockout models have been developed to further understand how specific genes might regulate acute pancreatitis. The method requires precise removal of the specific gene of interest and replacing it with a mutant gene that is inactive, altered or irrelevant. Rinderknecht [18] first commented that the severity of the local disease and the systemic complications may increase due to the inappropriate activation of the immune system. In recent years, there has been accumulating evidence demonstrating that synthesis and release of proinflammatory cytokines and chemokines influence the local injury and the systemic dispersion of the inflammation. Gene knockout models may be used to examine the effects of cytokine and chemokine mediators as well as their receptors in the pancreas and distant organs. It has been shown that the deletion or alteration of a single gene does not entirely prevent injury in the pancreas or remote organ. This is indicative of many redundancies of ligands and receptors characteristic of the cytokine and chemokine families. Gene knockout models have been used to determine the function of several specific targeted genes such as interleukin (IL)-1 and tumour necrosis factor-α [70–73], IL-6 [74], IL-10 [75], chemoattractant cytokine receptor-1 [76], neurokinin-1 receptor [77,78], intercellular adhesion molecule-1 (ICAM-1) [79–81], metallothionein-1 [82], cathepsin B [83], mouse α2-macroglobulin and murinoglobulin [84], complement factor C5a [85,86], granulocyte-macrophage colony-stimulating factor [20] and phospholipase A2 [87].

Knockout models are increasingly used to examine the cause and course of acute pancreatitis. The advantage of the gene knockout models is that by deleting the specific gene of interest, its function or effect can be revealed or clarified. Furthermore, the use of pharmacological manipulations which often cause side effects can be avoided. This allows investigation of the effects of a specific cytokine to be carried out. In general, the gene knockout model is time-consuming, expensive and complex to perform. Another disadvantage is that the specific gene alteration from the time of its conception could mean that other protein expressions may result to compensate for the mutation. In addition, the mutation may stimulate unforeseen phenotypic changes if a gene is expressed in different tissues. On the other hand, the expression of two genes may overlap and the alteration in a single gene might mask an abnormal phenotype. Any extrapolation of experimental data from gene knockout models to humans is therefore difficult.

Immune-mediated model

Immune-mediated models of acute pancreatitis may be induced by both non-invasive and invasive means, usually involving pancreatic intraductal infusion of a variety of agents. Thal and Brackney [88] first described an immunologically induced acute pancreatitis model by infusing bacterial toxin of Escherichia coli and meningococci into the pancreatic duct of goats and rabbits. This produced a local Shwartzman reaction in the pancreas. The authors administered the same toxin 24 h later which led to acute haemorrhagic and necrotizing pancreatitis. None of the animals survived within 4–24 h after the second doses [88]. In a separate study, Thal [89] reported other varieties of acute pancreatitis associated with an Arthus phenomenon which involved sensitizing rabbits by intravenous or subcutaneous administration of ovalbumin. The degree of pancreatitis ranged from acute non-fatal interstitial pancreatitis to severe pancreatic necrosis. Thal [89] observed that both types of experimental acute pancreatitis resembled the interstitial and necrotic forms seen in humans. The morphological studies of these models have suggested that vascular factors induced by both the Shwartzman and Arthus reactions have been identified as playing a major role in the pathophysiological pathways of this model of experimental acute pancreatitis.

Acute pancreatitis has also been induced by an intraperitoneal injection of foreign serum in mice. This model is characterized by acinar cell necrosis, fat necrosis, hyperamylasaemia and inflammatory response syndromes [90,91]. Nevalainen [92] described another model of acute necrotizing pancreatitis by injecting fresh rabbit serum either intraperitoneally or into the rat pancreatic duct. Features of early membrane impairment resulting in osmotic damage, death and necrosis of affected acinar cells were observed [92]. Other immunology-based models have been documented [93,94]. These include intraductal administration of anti-acinar cell antiserum to rats causing a progressive inflammatory process leading to severe necrosis within 16 h of induction. A spontaneous model of autoimmune acute pancreatitis in the MRL/MP strain of mice has also been described [95]. Histological features include severe inflammatory changes and extensive acinar tissue necrosis, which appear to be mediated by cellular autoimmune mechanisms, in particular, CD4+ cells [95]. Acute pancreatitis in this model may be induced in genetically different animals by the transfer of spleen cells from the affected animal [95].

Immune-mediated models are not recommended for the study of acute pancreatitis. These models are challenging to set up in the laboratory, time-consuming, have limited reproducibility, are costly, and the clinical relevance is unclear [96]. The high early mortality rate makes investigation of pathogenesis or treatment options difficult to assess. Another major disadvantage of this model is the development of secondary diabetes due to the involvement of the islets of Langerhans [97]. One possible application of this model is in the field of drug- or toxin-induced acute pancreatitis [97].
**Diet-induced model**

Lombardi et al. [98] developed a diet-induced model of acute pancreatitis by inducing severe acute necrotizing pancreatitis in young female mice fed a choline-deficient diet containing ethionine (CDE diet). The synergistic effect of choline deficiency and ethionine administration may be manipulated to achieve the desired mortality rates. Animals fed with choline-sufficient diets and ethionine 0.5% leads to a mortality rate approaching 10%. A choline-deficient diet without ethionine did not cause any mortality nor acute pancreatitis [99]. By modifying the feeding protocol established by Lombardi et al. [98], Gilliland and Steer [100] were able to decrease the severity of diet-induced acute pancreatitis. The CDE diet was reduced in terms of amount (3 g per mouse) and duration (24 h). The exposure to CDE diet feeding was performed before and after 24 h of fasting. From this modification, a mortality rate between 50% and 60% resulted. Glucagon has been shown to reduce the mortality rate and severity of acute pancreatitis if administered prior to a CDE diet. Pancreatic polypeptide also decreased mortality rate and severity of acute pancreatitis. This model therefore allows gradation of the severity of acute pancreatitis and mortality for therapeutic trials or cell biological studies.

The advantage of the diet-induced model is that it is perhaps the simplest system to establish for the investigation of acute haemorrhagic pancreatitis. It is also cheap, highly reproducible and no surgical procedure is involved. Furthermore, by restricting the period of feeding, the mortality rate can be controlled at any desired level between 0% and 100% [101]. The model is suitable for studying the pathophysiological aspects of the disease and the potential benefit of experimental treatment by measuring survival, biochemistry, histology, alterations in haematocrit, pH and blood gases [102]. In comparison to the mild and oedematous varieties of acute pancreatitis produced by the caerulein model, this diet-induced model produces a haemorrhagic and necrotizing appearance of the disease with a lethal course. The inflammatory lesions in these models are homogeneously distributed. The model is substantially defined and reliably produces severe necrosis that approximates human acute pancreatitis. It has been demonstrated that both the gross and histological appearance of the pancreatic and peripancreatic inflammation, in addition to the clinical and biochemical course of diet-induced acute pancreatitis, reflect the human disease. The presence of ascites, acidosis, hypoxia and hypovolaemia can be found in this diet-induced model as well as in human acute pancreatitis.

Although the morphological alterations have many similar features between mouse and man [103], the relevance to the aetiology of human acute pancreatitis is doubtful. The model produces 80–100% mortality after 2–8 days. The mortality, however, may be related to causes other than acute pancreatitis. Independent of acute pancreatitis, animals also have changes in the liver and central nervous system which contribute to multiple organ failure.

Furthermore, the diet-induced model is species-specific and may be used only in mice, whose small size causes considerable technical limitations. The model is also sex-specific. In contrast, male mice are resistant to the induction of acute haemorrhagic pancreatitis by a choline-deficient diet containing DL-ethionine [104]. Male mice with a body weight over 20 g usually show a less severe acute pancreatitis with a much lower mortality rate [49]. The administration of oestrogen increases the susceptibility of male mice to acute pancreatitis [105]. The onset of the acute pancreatitis is variable and careful monitoring is required to ensure that the intake of the CDE diet is the same in different experimental groups [99,101].

**L-Arginine model**

Another non-invasive rat model of acute pancreatitis was developed by Mizunuma et al. [106]. This model produces acute necrotizing pancreatitis by administration of a large dose of L-arginine (Arg). The mortality is 2.5% [107]. The mechanism by which Arg causes acute pancreatitis is currently unknown. It has been proposed that oxygen free radicals [108–112], nitric oxide [113], and inflammatory mediators [111,114–116] may be involved in the development of this disease. Hegyi et al. [107] noted that the weight of the pancreas almost doubled within 24 h. Under electron microscopy, Kishino et al. [117] noticed that degeneration began with the disarrangement of the rough endoplasmic reticulum in pancreatic acinar cells. Initial distension of the endoplasmic reticulum was followed by disarranged rough endoplasmic reticulum and degraded zymogen granules. This was followed by necrosis of acinar cells with leukocyte and fibroblast infiltration.

The single Arg injection model has been modified with varying results. Higher doses of >500 mg/100 g bm can induce death within a few hours [118]. Repeated dosing can increase the proportion of acinar cell necrosis. A single dose of 500 mg/100 g bm causes necrosis in up to 70–80% of the pancreatic acinar cells within 3 days, which increases to 90% with three further doses over 10 days [118]. Reduced doses over a longer time period can also be used to delay the time of onset of acute pancreatitis [119].

In 2004, Hegyi et al. [107] characterized the early and late phases of the Arg-induced model in the rat. The early phase of acute pancreatitis is characterized by acute pancreatic inflammation with intercellular oedema infiltration of leukocytes, capillary dilatation and microfocal parenchyma necrosis. The acinar cells surrounding the Langerhans inlets remained intact. Prominent adipose tissue deposition was seen at the

---

**Models for acute pancreatitis**

271
end of the first week. This adipose tissue accretum is an indication of atrophy in affected areas and may represent hypertrophy and hyperplasia in unaffected areas. The deaths of the rats occurred between days 1 and 5 following acute pancreatitis induction, with no mortality in control groups.

The benefits of the Arg-induced model include its high reproducibility and its ability to achieve selective dose-dependent pancreatic acinar cell necrosis. Due to the dose and time dependency of the effects of Arg, this model is very good for investigating the early and late phases of acute pancreatitis. A smaller dose of Arg is useful for characterizing the regenerative processes of acute pancreatitis. Long-term administration of Arg, however, results in chronic pancreatitis [119,120]. This model is also useful for investigating the insulo-acinar axis. In a clinical situation, the circulatory, pulmonary, renal and hepatic failure (multi-organ failure) significantly affects the morbidity and mortality of acute pancreatitis [121–123]. Thus, this model may be suitable for researching extra-pancreatic organ damage and its mechanisms.

**Invasive experimental models**

The highly variable severity of acute pancreatitis associated with the non-invasive models as well as the ill-defined temporal relationship of onset has led to the development of invasive models to overcome these problems (Table II). In addition, the aetiology of the non-invasive models has minimal relevance to the clinical situation. Most invasive animal models of acute pancreatitis simulate mechanisms relevant to gallstone-induced acute pancreatitis observed in clinical situations.

**Closed duodenal loop model**

Experimental acute pancreatitis in animals may be produced by creating a closed duodenal loop (CDL). This technique involves surgically closing above and below the duodenal papilla while bile is diverted into the jejunum via an implanted cannula. The model was first developed by Pfeffer et al. [124] in an attempt to improve the extensive variability experienced when using the retrograde duct-injection models. Evidence of oedema, haemorrhage and necrosis of the pancreas developed within 9–11 h. No inflammation or fat necrosis was evident. Pfeffer et al. [124] concluded that the vascular factors influenced the formation of tissue necrosis. The CDL model is more representative as a model of pancreatic necrosis than as a model of pancreatic inflammatory disease.

This model has since undergone a number of modifications and has been employed in various animal species. In a study by Byrne and Joison [125], it was shown that acute haemorrhagic pancreatitis may be prevented by inserting antibiotics in the duodenum after a CDL had been performed. This suggests that the bacteria may contribute to the pathogenesis of acute haemorrhagic pancreatitis [125]. The study by Nance et al. [126] however, demonstrated that the surgical creation of a CDL in germ-free dogs still induced acute pancreatitis. Furthermore, primates develop acute pancreatitis only if the CDL is combined with stimulation of the pancreas [127]. Hence, differences between different animal species must be taken into account when considering this model. It is now clear that the mechanism of acute pancreatitis is triggered by the reflux of duodenal contents into the pancreatic duct, and the pathogenesis in the CDL animal model is based on this principle.

Nevalainen and Seppa [128] also conducted a modification of CDL in rats. An intraduodenal tube was placed prior to ligating the duodenal loop in order to restore the continuity of the gastrointestinal tract. Within 24 h, acute haemorrhagic pancreatitis was visible in the animals. The findings support the view that reflux of the duodenal contents into the pancreatic duct is an important early pathogenetic mechanism in the development of acute pancreatitis. Chetty et al. [129] performed another modification of the technique by instilling infected bile into the closed loop under pressure, at a known time, in the rat. This resulted in the production of a lethal haemorrhage, periductal abscess formation and fat necrosis. The model of Chetty et al. [129] produces a consistently severe acute pancreatitis in comparison with the model produced by Nevalainen and Seppa [128].

Since the permanent ligation of the duodenum often caused death of the animal within a short period, some investigators have attempted temporary ligation of the duodenum in rats [130,131]. This allowed them to examine the progression of acute pancreatitis at an extended time and to investigate potential therapies. In a study by Orda et al. [131], the authors injected a mixture of sodium taurocholate and trypsin into a temporary CDL to induce acute pancreatitis in rats. They found that the acute pancreatitis induced was mild, associated with a mortality rate of 45%. Recent studies have demonstrated a number of treatments that have an effect on both the course and severity of acute pancreatitis. These include protease inhibitors [131,132], trypsin inhibitors [134–136], somatostatin [137], prostaglandin E₁ [138], vasopressin [139] and dextran 40 [140].

The CDL model has helped address some of the issues regarding the aetiology of acute pancreatitis, specifically the role of duodenal reflux in the pathogenesis of the disease in humans. The model is advantageous because of its simplicity and reproducibility. It is suitable for experiments on small animals such as rats and thus is relatively economical. Disadvantages of this model include the need for surgical intervention, the non-physiologic pressure levels created in the closed duodenal compartment and the pancreatic duct, and the controversial role of bacterial
<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Clinical relevance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed duodenal loop (CDL)</td>
<td>• Relatively simple</td>
<td>• Requires surgical intervention, the non-physiologic pressure levels created</td>
<td>• Closely resembles the few patients who develop acute pancreatitis in association with afferent loop obstruction after Billroth II gastrectomy</td>
<td>108–110, 112–115</td>
</tr>
<tr>
<td></td>
<td>• Reproducible</td>
<td>• Not widely used</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Useful for studying the role of duodenal reflux in the pathogenesis of</td>
<td>• Requires surgical intervention, the non-physiologic pressure levels created</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>acute pancreatitis in humans</td>
<td>• Not widely used</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Suitable for experiments on small animals (i.e. rats) and thus economical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antegrade pancreatic duct perfusion</td>
<td>• Useful for studying early pathophysiology and subsequent progression of</td>
<td>• Complex (ductal cannulation and perfusion)</td>
<td>• Aetiology and morphological changes closely resemble the human situation</td>
<td>126–129</td>
</tr>
<tr>
<td></td>
<td>acute pancreatitis</td>
<td>• Often requires large animals (i.e. cats) which are expensive and difficult</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Reliable</td>
<td>to maintain</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Reproducible</td>
<td>• Pancreatitis severity may be varied within well-defined time intervals according to the volumes and perfusion pressures</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Ideal for assessing potential therapeutic agents (i.e. cholecystokinin antagonists, somatostatin, fibrinolytic agents and cytokine antagonists)</td>
<td>• Reproducible</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Perfusion of an isolated segment of the rat pancreatic duct with bile salt increases its permeability; therefore smaller and cheaper animals can be used (i.e. rats)</td>
<td>• Pancreatitis severity may be varied within well-defined time intervals according to the volumes and perfusion pressures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliopancreatic duct injection</td>
<td>• Suitable for studying systemic issues of acute pancreatitis</td>
<td>• Technically challenging to control constant pressure recordings and hence produce a standard degree of injury</td>
<td>• Clinical and pathogenetic relevance unclear</td>
<td>131, 136–138</td>
</tr>
<tr>
<td></td>
<td>• Useful for studying pseudocyst formation, pancreatic abscess and fatty tissue necrosis</td>
<td>• Difficult to establish the degree of variation regarding tissue necrosis and survival in animal groups</td>
<td>• Limited evidence to support the role of bile reflux in the human condition where the bile concentration in the general tissue is higher than the pancreatic tissue concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Reproducible model for creating a severe, rapidly evolving, and lethal variety of acute hemorrhagic pancreatitis</td>
<td>• Surgery is restricted to simulate the type of obstruction that would take place during the passage of gallstones via the papilla of Vater</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II. Summary of the advantages, disadvantages and clinical relevance of invasive experimental models of acute pancreatitis.
<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Clinical relevance</th>
<th>References</th>
</tr>
</thead>
</table>
| Combination of secretory hyperstimulation with minimal intraductal bile acid exposure | - Combined action of low-dose intraductal GDOC with intravenous caerulein causes acute pancreatitis more reliably than induction by either component alone  
- Accelerated detergent effect is not elicited when using very low dose of GDOC (5 or 10 mmol/l); the bile salt is dispersed throughout the periphery of the pancreas  
- Relatively easy to perform  
- Reproducible  
- Relatively inexpensive  
- Mortality rates vary between 6% and 42% at 24 h  
- Pancreatic cell injury is progressive over at least 24 h  
- Produces homogeneous injury of intermediate rate of onset suitable for scoring and testing treatments  
- Pancreatic injury can be manipulated by controlling the amount and time of infusion of intraductal low-dose GDOC | - High concentrations of bile acids produce extensive acinar necrosis too rapidly and the resulting lesions show substantial heterogeneity  
- Applying very low concentrations of GDOC (5 or 10 mmol/l) produces non-remarkable acute pancreatitis, with no death | Homogeneous injury has a moderately severe acute pancreatitis that impairs all regions of the gland, resembling human acute pancreatitis | 139 |
| Vascular-induced Microcirculatory disturbance | | | | 149, 150, 152–156 |
| | - Acute haemorrhagic necrotizing pancreatitis can be reproduced by using microspheres of diameters < 20 μm  
- Induction of hypovolaemic shock significantly lowers pancreatic blood flow, thus may potentially be used to study pancreatic damage in cardiac surgery  
- Useful for studying coagulopathy and thrombosis of microvessels caused by acute pancreatitis  
- Allows the study of endothelin receptor antagonists (i.e. ET-RA, PAF-RA, ICAM-1-AB); ET-RA is most effective | - Maintaining the normality of other organs during the cannulation of pancreatic vessels is difficult | Microcirculatory disturbance models increase capillary permeability, similar to clinical situation | |
<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Clinical relevance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>- Vascular-induced models are relatively inexpensive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pancreatic artery occlusion</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Limited reproducibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pancreatic venous occlusion</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Not suitable for investigating local changes in the pancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Not easily reproducible</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overall</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Vascular-induced models do not reliably induce equivalent severity of acute pancreatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Reproducibility sometimes limited due to extensive collateral network of pancreatic circulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Require large experimental animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Surgical procedures are time-consuming</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Minimal histological changes of acute pancreatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- May eventually progress to chronic pancreatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemia/reperfusion</td>
<td>- Hoffman’s model allows complete and reversible interruption of arterial blood supply to the pancreas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Intravital fluorescence microscopy enables the parameters of microcirculation during the entire reperfusion period to be assessed repetitively and quantitatively</td>
<td></td>
<td></td>
<td>154, 163</td>
</tr>
<tr>
<td></td>
<td>- Current models – incomplete ischaemia and inability to quantitate the remaining blood supply to the pancreas; irreversible ischaemia, making it unsuitable for reperfusion studies; and complete and reversible ischemia, limited to <em>ex vivo</em> models</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Quantitative analysis of post-ischaemic reperfusion failure at the microcirculation level is difficult to achieve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Various methods, i.e. methylene blue or India ink injection, radioactive microspheres injection, light and electron microscopic analysis of microangiographic architecture or laser Doppler flowmetry cannot be used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duct ligation</td>
<td>- Avoids artificial drug usage that may produce unwanted systemic effects and the theory relating to clinical acute biliary acute pancreatitis with biliary pancreatic reflux</td>
<td></td>
<td></td>
<td>108, 113, 171, 177, 178, 184</td>
</tr>
<tr>
<td></td>
<td>- Cannot induce acute pancreatitis by surgical ligation of pancreatic duct alone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Ligation of the common bilio-pancreatic duct in the rat causes a clinical syndrome resembling the multiple organ failure seen in humans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>Advantages</td>
<td>Disadvantages</td>
<td>Clinical relevance</td>
<td>References</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------</td>
<td>------------------</td>
</tr>
</tbody>
</table>
| Duct-ligated Opossum/possum  | • Ligation of the pancreatic duct alone, bile and pancreatic duct separately, or the common biliopancreatic duct leads to severe acute pancreatitis  
  • Mortality rates approach 100% within 2 weeks of induction  
  • Useful for investigating pathophysiology of biliary acute pancreatitis  
  • Necrosis is the primary mechanism; apoptosis is only present in the early stages of acute pancreatitis  
  • Well characterized  | • Most laboratory animals develop chronic lesions in the pancreas characterized by atrophy and apoptosis of acinar and ductal tissue, but not significant necrosis or inflammation  
  • Technically difficult to perform  
  • Expensive  
  • Limited reproducibility  
  • Analogous to chronic pancreatitis  | • Main clinical correlation of duct ligation-induced pancreatitis is the acute pancreatitis seen after Polya gastrectomy  | 179–182, 184, 186 |
| Opossum                      | • Closely simulates the mechanical events that occur during or after gallstone passage  | • Co-existing duodenal wall necrosis, and pancreatic peritoneal sepsis often add complexity to the model; this complicates interpretation of results  | • The arrangement of the pancreatic and bile ducts come together and are controlled by an SO complex, resembling those of humans  |                  |
| Possum                       | • Usefulness for studying the motility of the sphincter of Oddi (SO); it functions as a variable resistor in modulating bile and pancreatic secretion  
  • Suitable for investigating the role of SO motility in other forms of acute pancreatitis, i.e. gallstone pancreatitis, and pancreatitis secondary to alcohol, scorpion envenomation, organophosphate poisoning, and octreotide treatment  
  • Does not require gastroduodenostomy  
  • Readily available  | • Responses to hormonal and drug stimuli are similar to humans  |                                                                                     |                  |
infection. The CDL model is thought to closely resemble the few patients who develop acute pancreatitis in association with afferent loop obstruction after Billroth II gastrectomy. On the whole, the CDL technique has not been widely used due to the associated limitations and the availability of other models [141].

Antegrade pancreatic duct perfusion model

The antegrade duct perfusion model has played a significant role in the understanding of the underlying mechanisms in the initial induction of acute pancreatitis. The main pancreatic duct can be made permeable by the perfusion of glycodeloxycholic acid (GDOC) along the main pancreatic duct. Other methods include the administration of intragastric ethanol, the stimulation of pancreatic secretion into an obstructed duct, or causing acute hypercalcaemia. Thereafter, activated pancreatic enzymes are perfused via the main pancreatic duct using a cannula implanted into the duct in the tail of the pancreas. The perfusion pressure is maintained at 20 cm H2O, which is less than the maximal secretory pressure in the pancreas of the cat [142–144]. Acute oedematous pancreatitis develops if activated pancreatic enzymes are perfused following early induced changes in ductal permeability [142]. The initial mild acute oedematous pancreatitis may be converted into an acute necrotizing pancreatitis by simultaneous infusion of the stable prostaglandin analogue 16-16-dimethyl-prostaglandin E2 [143]. This low-pressure duct perfusion model causes acute pancreatitis in cats resembling human changes and may be ideal for the study of severe necrotizing disease [142–144]. Olazabal et al. [145] reported that perfusion of an isolated segment of the rat pancreatic duct with bile salt increased its permeability. Thus, this technique can be used on smaller animals, making it more economical.

The advantages of the pancreatic duct perfusion model are its reliability and reproducibility. The severity of the acute pancreatitis may be varied within well defined time intervals according to the volumes and perfusion pressures. The aetiology and the morphological changes closely resemble the human situation. This model is suitable for studying the early pathophysiology and subsequent progression of the disease. It is the ideal model to assess potential therapeutic agents, including CCK antagonists, somatostatin, fibrinolytic agents and cytokine antagonists (see references/review in Bilchik et al. [146]). Furthermore, this model allows the ability to modify duct obstruction to study chronic pancreatitis.

The major disadvantage of this model is its complexity (ductal cannulation and perfusion). Other disadvantages include the need to use large animals such as cats which are expensive and difficult to maintain. However, once standardization is achieved, it remains one of the most suitable models to assess the underlying pathophysiology of severe acute pancreatitis and the effects of any therapeutic intervention.

Biliopancreatic duct injection model

Various compounds have been infused into the pancreatic duct to induce acute pancreatitis [147–151]. Following a duodenotomy, a retrograde injection of bile salts (with or without activated pancreatic enzymes) into the pancreatic duct at the ampulla leads to severe acute pancreatitis. The severity of the disease can be manipulated by changing either the pressure or the concentration of bile salt used. Acute severe pancreatitis develops within 2–24 h and is characterized by oedema, necrosis and haemorrhage. Almost any form of detergent injected into the pancreatic duct under pressure will cause acute pancreatitis in laboratory animals such as dogs [152] and rats [147,153,154]. One of the best standardized compounds to use to develop acute pancreatitis is sodium taurocholate. Infusion of 0.2 ml/kg of 3%, 4.5% or 5% solution induced acute haemorrhagic pancreatitis with 72-h mortality rates of 24%, 71% and 100%, respectively [147,153,154]. This model is appropriate for studies of systemic issues.

The retrograde injection of bile salts into the pancreatic duct of animals is an easy, effective and reproducible model for creating a severe, rapidly evolving and lethal variety of acute haemorrhagic pancreatitis. This model may also be used to study pseudocyst formation, pancreatic abscess and fatty tissue necrosis. Haemorrhagic and necrotizing forms of acute pancreatitis have been reproducible for therapeutic studies when specific, low concentrations of bile salts [155,156] were used.

An important limitation of the biliopancreatic duct injection model is its clinical and pathogenetic relevance. There is limited evidence to support the role of bile reflux in the human condition where the bile concentration in the general tissue is higher than the pancreatic tissue concentration [157]. It is also technically challenging to control constant pressure recordings and hence produce a standard degree of injury. Another disadvantage of this technique is the injection pressure with which the solution is applied to cause severe acute pancreatitis [158]. Solutions such as normal saline can produce acute pancreatitis when injected to high pressures [159]. Current studies have tried to overcome this artefact [160] by applying very low perfusion pressures and defined injection intervals to yield reproducible results [155]. In addition, it is critical to establish the degree of variation regarding tissue necrosis and survival in the animal groups. Another limitation is that surgery is restricted to simulate the type of obstruction that would take place during the passage of gallstones via the papilla of Vater.
Combination of secretory hyperstimulation with minimal intraductal bile acid exposure model

A new model has been characterized by Schmidt et al. [155] to improve the limitations of acute pancreatitis models described above. This model involves a combined action of low-dose intraductal glycodeoxycholic acid (GDOC) with intravenous caerulein. The combination of both agents causes acute pancreatitis more reliably than induction by either component alone. This model produces a homogeneous injury with a moderately severe acute pancreatitis that impairs all regions of the gland, similar to human acute pancreatitis.

The disadvantage of this model is that a high concentration of GDOC (34 mmol/l) does not produce desirable results. It causes substantial acinar necrosis to form too quickly and the resulting lesions show considerable heterogeneity. On the other hand, using a very low dose of GDOC (5 or 10 mmol/l) is more advantageous because the accelerated detergent effect is not elicited. Furthermore, the bile salt is dispersed throughout the periphery of the pancreas. However, the limitation of applying very low concentrations of GDOC is that the acute pancreatitis produced is not remarkable, with no death.

This new model is advantageous because it is easy to perform, reproducible and inexpensive. The mortality rates vary between 6% and 42% at 24 h. In addition, the pancreatic cell injury produced is progressive over at least 24 h. The model produces homogeneous injury of intermediate rate of onset, which can be reliably scored for comparisons and enable treatment to be easily modulated. Pancreatic injury may be manipulated to become more severe and death is advanced by further increasing the amount and time of infusion of intraductal low-dose GDOC.

Vascular-induced model

Clinical syndromes associated with microvessel abnormalities are a rare cause of acute pancreatitis in humans. Acute pancreatitis may occur infrequently in polyarteritis nodosa, and in various coagulopathic states associated with microvascular thrombosis [161]. Ischaemia from occlusion of the major arterial or venous supply of the pancreas may also be a rare cause of acute pancreatitis in humans. Various models have been developed to examine the vascular mechanism that produces acute pancreatitis.

Microcirculatory disorder is the major cause of death in the course of severe acute pancreatitis [162]. Experimental evidence has shown that microcirculatory disturbances in this disease not only initiate tissue damage locally, but also in distant organs including the colon, liver, lungs and kidney [163]. Hence, microcirculatory disorders are thought to be crucial in the development of acute pancreatitis-associated multiple organ dysfunction syndrome [164].

In 1962, Pfeffer et al. [165] found that intravascular injection with 10–20 µm microsphere diameter caused minimal oedema; 20–40 µm induced moderate oedema, parenchymal necrosis and inflammation; and 10–20 µm induced acute haemorrhagic necrotizing pancreatitis. There was also a significant increase in serum amylase levels. The results of this study were reproducible. A disadvantage of inducing acute pancreatitis by disturbing the pancreatic microcirculation is the difficulty in maintaining the normality of other organs during the cannulation of pancreatic vessels. The advantage of this method is that acute haemorrhagic necrotizing pancreatitis can be reproduced by using microspheres of diameters below 20 µm. This is particularly useful in the study of coagulopathy and thrombosis of microvessels caused by acute pancreatitis.

Other models have been developed to simulate the impairment of pancreatic inflow and outflow or the disturbances of the pancreatic microcirculation. In 1974, Lefer and Spath [166] induced hypovolaemic shock in dogs by bleeding them to a mean arterial pressure of 45 mmHg for 3 h. Electron microscopy showed that the lysosomes and zymogen granules became enlarged, and cathepsin D increased by 27-fold. Barzilai et al. [167] also used dogs to induce hypovolaemic shock. The dogs were bled for a period of 3 h to a higher mean arterial pressure of 60 mmHg. The authors found oedema and acinar cell necrosis in the pancreas. They also found that hypovolaemic shock significantly lowered pancreatic blood flow. The model may potentially be used to study pancreatic damage in cardiac surgery [168]. This model is now specific and produces multiple systemic hypotensive changes.

There is experimental evidence that microcirculatory disturbances involve not only decreased capillary blood flow but also enhanced leukocyte–endothelial interaction and increased capillary permeability [169]. High capillary permeability, in particular, represents a relevant clinical problem. Several studies have attempted blocking vasoactive mediators such as endothelin (ET-1), platelet-activating factor (PAF) and intercellular adhesion molecule-1 (ICAM-1), in the same model of severe acute pancreatitis to study their effects. Eibl et al. [169] noted that endothelin receptor antagonist (ET-RA), PAF receptor antagonist (PAF-RA) and intercellular adhesion molecule-1 antibody (ICAM-1-AB) improved microcirculation in acute pancreatitis, confirming previous studies. Moreover, the authors found that ET-RA is the most effective in reducing microcirculatory disorders, particularly in decreasing capillary leakage. Therefore, endothelial receptor blockade could be useful for a clinical trial to attenuate capillary leakage in individuals with acute pancreatitis.
In addition, various studies have been performed to occlude pancreatic arteries. In 1986, Spormann et al. [170] occluded the coeliac axis and the superior mesenteric artery for 20 min and found a three-fold increase in serum amylase after 4 h. They did not find any histological changes in the pancreas. In another study, Pfeffer et al. (165) performed a permanent ligation of the superior pancreaticoduodenal artery and observed a rise in pancreatic enzymes and parenchymal necrosis in the head region of the pancreas. The main disadvantage of this model is its limited reproducibility. Redha et al. [171] also reported an induction of acute haemorrhagic pancreatitis by partially occluding the arterial supply to the pancreas of the rat. This technique required a laparotomy and delivering the spleen into the wound. A microcannula was used to isolate a distal branch of the splenic artery. Polystyrene microspheres were introduced into the artery. The cannula was then removed and the vessel ligated. Alterations of the pancreas can be identified within 30 min of induction by immunohistochemical techniques [171]. Within 24 h, there was extensive pancreatic oedema, inflammatory cell infiltration, haemorrhage and fat necrosis [171]. An advantage of the pancreatic artery occlusion model is relative preservation of islet cell function [97]. On the whole, pancreatic artery occlusion models are not useful for investigating acute pancreatitis. In a clinical setting, acute pancreatitis caused by occlusion of a main artery is not a common event. It is sometimes observed in patients with arteritis.

A number of studies have also been performed to study the occlusion of pancreatic veins. Pfeffer et al. [165] investigated the permanent occlusion of the pancreatic outflow in dogs. They injected microspheres of 20–40 μm diameter via the superior pancreaticoduodenal vein into the pancreas. The pancreas and serum amylase remained normal. There was also no evidence of morphological changes in the pancreas. In contrast, Sjovall et al. [172] found a four-fold increase in amylase concentration in rats after ligating the splenic and superior pancreaticoduodenal vein. They also observed oedema and interstitial haemorrhage in the pancreas 25 h after ligation. Shibayama et al. [173] found mild oedema and haemorrhage in the pancreas within 24 h of partial or complete venous obstruction in rats. The major disadvantage of occluding pancreatic veins is its unsuitability for investigating local changes in the pancreas. Furthermore, partial vein occlusion is not easily reproducible. The pancreatic venous occlusion model may be used to study the pathophysiology of acute pancreatitis in acute splenic vein or portal vein thrombosis.

The advantage of the vascular model is that it is relatively inexpensive. The model does not reliably induce equivalent severity of acute pancreatitis. The reproducibility of this model is sometimes limited because of the extensive collateral network of the pancreatic circulation. It is however, important for studying microcirculatory disturbances that may take place in acute pancreatitis as a result of coagulopathy and microvascular thrombosis [161]. The disadvantages of this model are the requirements of large experimental animals and time-consuming surgical procedures. Although temporary arterial occlusion shows a transient rise in circulating pancreatic enzymes, histological changes of acute pancreatitis are minimal. Overall, the vascular-induced model has not been particularly useful in investigating acute pancreatitis. There is evidence [174] that this model may ultimately advance to chronic pancreatitis and may be a useful tool to investigate the chronic form of this disease.

Ischaemia/reperfusion model

Both clinical and experimental studies have shown that ischaemic injury plays an important part in the pathogenesis of acute pancreatitis. Ischaemic injury to the pancreas is known to occur in various clinical settings including cardiopulmonary bypass [175], haemorrhagic shock [168] and transplantation of the gland [176]. Studies have shown that there is correlation between the impairment of microcirculation in human pancreas with the degree of ischaemic injury [177], and microvasculature presents the primary target of reperfusion injury after ischaemia [178]. In recent years, animal models have been developed to investigate the ischaemia/reperfusion of the pancreas.

In 1995, Hoffman et al. [179] developed a model to study the microcirculation of the pancreas in the rat after complete (interruption of arterial blood supply to the pancreas) and reversible ischaemia of the pancreas using intravital fluorescence microscopy. Blood supply for complete ischaemia of the pancreas was impaired by isolating arteries from surrounding tissue of gastroduodenal artery, left gastric artery, splenic artery and caudal pancreaticoduodenal artery. Complete but reversible ischaemia of the pancreas was induced by occluding the four vessels using microvascular clips. The clips were taken out 30 min, 1 h or 2 h after ischaemia to produce reperfusion.

The characteristics noted following ischaemia/reperfusion in the pancreas were similar to those observed in the sodium taurocholate-induced acute pancreatitis model [179]. The duration of ischaemia and of reperfusion is responsible for the severity of post-ischaemic inflammatory reaction. In addition, there was a rise in the concentration of serum amylase after 1 and 2 h of ischaemia [179]. Similar results were observed by another group after 2 h of ischaemia and 4 h of reperfusion [180]. Redha et al. [171] reported even higher serum amylase concentrations after ischaemia-induced acute pancreatitis. This may occur because of the extended time (27 h) of reperfusion. Under light and electron microscopy,
the histological changes resembled those seen in the caerulein-induced acute pancreatitis in the rat [181]. Ultrastructural changes after 2 h of ischaemia simulate acinar structural changes in pancreas grafts following transplantation in human [182,183].

The current models used to induce ischaemia have a number of restrictions involving reproducibility. These limitations include incomplete ischaemia and inability to quantitate the remaining blood supply to the pancreas; irreversible ischaemia, making it not suitable for reperfusion studies; and complete and reversible ischaemia, limited to ex vivo models [179]. Furthermore, quantitative analysis of the post-ischaemic reperfusion failure at the microcirculation level is also difficult to achieve. Various methods such as methylene blue or India ink injection, radioactive microspheres injection, light and electron microscopic analysis of microangiographic architecture or laser Doppler flowmetry cannot be used [184–186]. The advantage of the model developed by Hoffman et al. [179] is that it enables complete and reversible interruption of arterial blood supply to the pancreas. By using the intravital fluorescence microscopy, the parameters of microcirculation during the entire reperfusion period can be assessed repetitively and quantitatively [179].

**Duct ligation model**

Acute pancreatitis may be induced by ligating the distal bile duct at the level of the duodenum [187]. This creates an early development of acute pancreatitis, obstructive jaundice and cholangitis in the rat. The duct ligation model was developed in an attempt to resemble the clinical situation of gallstones, motility disorders of the sphincter, oedema and strictures at the papilla, tumours of the papilla, and parasites impacting the terminal biliopancreatic duct.

Surgical ligation of the pancreatic duct alone has not been successful in inducing acute pancreatitis. Most laboratory animals developed chronic lesions in the pancreas characterized by atrophy and apoptosis of acinar and ductal tissue, but not significant necrosis or inflammation [188]. The ligation of the pancreatic duct in rodents has become an established model for chronic pancreatic atrophy and is often used for studying pancreatic regeneration [189,190]. Ligation of this common biliopancreatic duct in the rat, however, causes a clinical syndrome resembling the multiple organ failure observed in man. These characteristics included pancreatic necrosis and haemorrhage, white cell infiltration and multiple microthrombi in the lung, stomach and kidney [191].

When Popper and Necheles [192] combined pancreatic duct ligation with the secretory stimulation, secretin in dogs, mild acute oedematous pancreatitis with fat necrosis was observed. Another study combining duct ligation with both secretory stimulation and minimal arterial blood produced a greater degree of severity of acute pancreatitis [193]. Due to its anatomical characteristics, the opossum has been used to produce severe acute haemorrhagic necrotizing pancreatitis. This laboratory animal has a pancreatic main duct which drains into a long common bile duct and both enter the duodenum at the papilla [194,195]. Ligation of the pancreatic duct alone, bile and pancreatic duct separately, or the common biliopancreatic duct leads to severe acute pancreatitis with mortality rates approaching 100% within 2 weeks of induction [196,197]. The American opossum model was frequently used to investigate the pathophysiology of biliary acute pancreatitis.

This model of duct obstruction in the opossum is useful because it closely simulates the mechanical events that occur during or after gallstone passage [198]. Using this model, acinar cell necrosis could be identified as being the early point of injury in acute necrotizing pancreatitis [194]. Studies have shown that in duct-ligated rats, the predominant mechanism of cell death is apoptosis [199]. In duct-ligated opossums, necrosis is the primary mechanism; apoptosis is only present in the early phase of acute pancreatitis [199]. Gukovskaya et al. [199] showed that the extent of pancreatic necrosis from duct-ligated opossums corresponds with the elevated levels of neutrophil infiltration. Neutrophils are thought to produce mediators such as hydrogen peroxide and oxygen that may be responsible for triggering necrosis [199]. Pancreatic outflow obstruction seems to be the critical pathophysiological event for the induction of acute pancreatitis [195]. It appears that bile reflux is not required for the pathogenesis of acute pancreatitis in this model.

It has been suggested that induced sphincter of Oddi (SO) dysfunction may be the cause of pancreatic duct obstructions [200]. A recent study using Australian brush tailed possums (Trichosurus vulpecula), has shown for the first time, that induced SO dysfunction when combined with stimulated pancreatic secretion produces acute pancreatitis. Chen et al. [201] demonstrated that transient obstruction of the SO produced by carbachol administration led to hyperamylasaemia and the histological changes correlated with acute pancreatitis. These results were similar to those induced in possums coupled with pancreatic duct ligation and CCK-8/secretin administration [201], a method well established in inducing hyperamylasaemia and acute pancreatitis in other species. Both these models required the simultaneous increase in pancreatic duct pressure with a combination of stimulated pancreatic exocrine to produce hyperamylasaemia and acute pancreatitis [201].

This newly described possum model has been well characterized. The model is useful for studying the motility of the SO because it functions as a variable resistor in modulating bile and pancreatic secretion [202]. The arrangement of the pancreatic and bile ducts come together and are controlled by an SO
complex, resembling those of humans. In addition, the responses to hormonal and drug stimuli are comparable to humans [203]. This model is also suitable for investigating the role of SO motility in other forms of acute pancreatitis such as gallstone pancreatitis, and pancreatitis secondary to alcohol, scorpion envenomation, organophosphate poisoning and octreotide treatment [200].

The duct ligation-induced model mainly correlates with the clinical acute pancreatitis seen after Polya gastrectomy. The advantage of the duct ligation model is that it avoids artificial drug usage which may produce unwanted systemic effects, as well as the theory relating to clinical acute biliary acute pancreatitis with biliary pancreatic reflux [197]. However, the complexity, technical difficulty, high cost, limited reproducibility and the analogy to chronic pancreatitis, have made the duct ligation model infrequently used for investigating acute pancreatitis. Chetty et al. [129] modified this model in the rat so that gastro-duodenostomy was not required. This model involves a plastic tube to traverse the CDL to restore gastrointestinal continuity. In comparison to the conventional model developed by Pfeffer et al. [124], the modified model is cheaper and more readily available. However, the co-existing duodenal wall necrosis and pancreatic peritoneal sepsis often add complexity to the model. This complicates interpretation of the results [129].

Conclusion

In view of the methodological restrictions of the established acute pancreatitis models for therapy investigation, many attempts have been made to develop, modify or combine available models to more closely resemble human acute pancreatitis. However, none of the existing experimental models are completely satisfactory at the present time. Therefore, comparison between the existing models is complex and interpretations of results from various studies are consequently difficult. The challenge is exposing the animals to the same degree of severity that triggers acute pancreatitis in man, such as alcohol, bile duct obstruction, pancreatic ischaemia, traumatic damage and various toxic substances. Although the current experimental models are not closely comparable to all the spectrum of human acute pancreatitis, previously reported studies should not be dismissed. Much of our current knowledge has stemmed from existing experimental models; they have contributed to our knowledge of mechanisms involved in early cellular events, pathogenesis and pathophysiology of acute pancreatitis. It has been shown that certain models are better suited for answering specific questions than others. Further understanding and standardization of these models may enable us to choose the most appropriate model for studying a given aspect of acute pancreatitis or for evaluating new treatment protocols. Thus, continued research refining the present experimental design is required in this area because the discovery of effective treatments for acute pancreatitis will be extremely beneficial.

Acknowledgements

Austin Hospital Medical Research Foundation Project Grant 2004.

References

K. H. Su et al.


