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REVIEW

# Do Animal Models of Acute Pancreatitis Reproduce Human Disease?

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## SUMMARY

Multiple animal models of clinical pancreatitis have been developed, however, limitations with respect to the pathobiology of human disease make it difficult to assess the validity of animal models. Although the growing availability of human pancreatic acinar cells are likely to provide information on early pancreatitis events that can be compared meaningfully with animal models, the relevance of animals to later events may be more difficult to obtain.

**Acute pancreatitis is currently the most common cause of hospital admission among all nonmalignant gastrointestinal diseases. To understand the pathophysiology of the disease and as a potential step toward developing targeted therapies, attempts to induce the disease experimentally began more than 100 years ago. Recent decades have seen progress in developing new experimental pancreatitis models as well as elucidating many underlying cell biological and pathophysiological disease mechanisms. Some models have been developed to reflect specific causes of acute pancreatitis in human beings. However, the paucity of data relating to the molecular mechanisms of human disease, the likelihood that multiple genetic and environmental factors affect the risk of disease development and its severity, and the limited information regarding the natural history of disease in human beings make it difficult to evaluate the value of disease models. Here, we provide an overview of key models and discuss our views on their strengths for characterizing cell biological disease mechanisms or for identifying potential therapeutic targets. We also acknowledge their limitations.** ***(Cell Mol Gastroenterol Hepatol 2017;4:251–262; http://dx.doi.org/10.1016/j.jcmgh.2017.05.007)***

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Although models of acute pancreatitis have been used for several decades, the extent to which they recapitulate the events in human beings remains unclear. The clinical course and limited pathologic material suggest that features of mild and severe pancreatitis generally are shared between these models and human beings.[1,2] Thus, mild and severe disease, and probably the new intermediate category of moderately severe disease, have similar courses and outcomes, although the time courses may differ, with common rodent models usually progressing more rapidly than human beings.

Some pancreatitis models are designed to examine issues related to the recognized causes of human disease and factors that might modulate disease severity. The major etiologies of acute clinical pancreatitis are gallstones, alcohol, and smoking. Procedure-related, especially after endoscopic retrograde cholangiopancreatography (ERCP), acute pancreatitis (PEP) represents an important subgroup for which specific early interventions appear to reduce disease incidence and severity.[3] Renal failure,[4] diabetes, and especially obesity are substantial risk factors for disease development and severity. Although an infrequent cause of pancreatitis, exposure to potent cholinergic agonists as seen with scorpion bites[5] and exposure to select insecticides[6,7] also can cause disease and may be mimicked by preparations that use supraphysiologic concentrations of toxins or neurohumoral agents to induce disease.[8] Infections with Coxsackie virus can cause acute pancreatitis in children, although the incidence is unclear; this response has been reproduced in animal models.[9] Known genetic factors vary in their clinical impact. For example, mutations in cationic trypsinogen alone can cause pancreatitis, but such mutations make a very small contribution to the clinical load of this disease. Mutations in other molecules such as serine protease inhibitor Kazal-type 1 and cystic fibrosis transmembrane conductance regulator appear to modify the risk of disease in the presence of other accepted factors, such as ethanol abuse. Although genetic models that reflect human variants have been generated, their value in reflecting clinical disease has been limited; we will not comment on these models further in this article.

Investigators striving to mimic these clinical etiologic factors in experimental models have focused on the underlying cellular mechanisms that are operative in these

***Abbreviations used in this paper:*** **CRISPR, clustered regularly interspaced short palindromic repeats; ERCP, endoscopic retrograde cholangiopancreatography; LPS, lipopolysaccharide; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PEP, procedure-related endoscopic retrograde cholangiopancreatography acute pancreatitis; PLA2, phospholipase A2; TRPV1, transient receptor potential vanilloid receptor 1.**

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disease etiologies. In this respect, animal models have been remarkably successful in characterizing intracellular processes that precede tissue injury.[10–12] Whether or not targeting these processes successfully in animal experiments predicts a similar beneficial effect in clinical trials remains another matter (Table 1).

## Animal Models of Clinical Acute Pancreatitis

Studies in experimental acute pancreatitis models are performed with several potential goals in mind. Most often is the wish to reproduce the mechanisms and processes underlying disease and/or examine therapeutic interventions, or use models to examine basic features of acute injury, inflammation, or tissue reconstitution. This means that the timing of an intervention needs to be relevant to the phase of disease. Models also can be used to examine the relationship among organs during the course of acute injury. For example, how does pancreatic injury affect the lungs or the kidneys? Models also can explore factors that directly cause disease, sensitizers that affect the risk of developing disease or modulate its severity, or both. One lesson learned from both studies of models and human pancreatitis is that sequential and overlapping pathologic responses underlie this disease.

Models of acute pancreatitis study the disease at the cellular and whole-animal level and in a range of species. Thus, isolated groups of pancreatic acinar cells long have been used to study basic cell physiology and more recently acute pancreatitis responses. Although they have the advantage of investigating responses in the absence of inflammation and changes in blood flow, their acinar cell phenotype disappears within hours of being placed in culture, making them useful for examining only early pancreatitis responses. Intact animal models of pancreatitis have been generated in many species including dogs, cats, guinea pigs, and even zebra fish.[13,14] Although many species have been used, rats and mice are used most commonly. This is because of their relatively low cost, the high reproducibility of rodent models, our potential to simulate the conditions we believe lead to human disease, and the growing efficiency in manipulating gene structure and function. Here, we focus on rodent models of acute pancreatitis and their potential relevance to human disease beginning with in vivo models and concluding with isolated cell preparations.

**Table 1.** Examples of Factors That Make it Difficult to Assess the Relevance of Acute Pancreatitis Models

| |
|---|
| Rodents |
| Inbred strains and strain differences |
| Variations in microbiome and diet |
| Potential differences in key regulatory/target proteins |
| Differences in preparations/experimental conditions |
| Incomplete information (time course, inflammatory responses, multi-organ effects) |
| Human beings |
| Inherent human genetic and epigenetic variation |
| Lack of knowledge of both how injurious factors cause disease and reproducing them in animal models |
| Paucity of tissue for histologic analysis and correlation |
| Incomplete information relating to the natural history of disease and organ reconstitution |

Although rodent models are used most often, their differences from human exocrine pancreas with regard to various factors, such as digestive enzyme content, should be recognized. Experimental pancreatitis preparations vary in their difficulty, reproducibility, and apparent relevance to clinical disease.[14] Because the clinical relevance of most models remains unclear, some studies have examined the efficacy of interventions using multiple models, which we view as wise.[15,16] Later, we comment on some of the key experimental pancreatitis models.

### *Cerulein-Induced Pancreatitis*

One of the oldest and most often used models of acute pancreatitis uses cerulein, a peptide orthologue of cholecystokinin, that when given in doses that are 10–100 times greater than a physiologic equivalent, causes mild acute pancreatitis in rats and a somewhat more severe variety in mice.[17,18] From 1 to 12 doses may be given hourly to induce disease. Although this also leads to lung injury, both the pancreatitis and lung injury are fully reversible within hours to a few days of the treatment. Mechanistically, this model is most similar to the pancreatitis caused by scorpion venom and cholinergic toxins that are thought to represent states of supraphysiologic neurohumoral stimulation. The model has been highly favored because of its strong reproducibility, simplicity, and the ease with which processes of intracellular protease activation can be studied.[19,20] It also has been extended to generate other forms of pancreatitis, such as the combination of cerulein with partial duct ligation, which allows simultaneous investigations of acute reversible changes and progressive fibrosis within the same organ.[21] As another example, when cerulein is given in repeated doses over time, the model can be converted to one that generates either severe pancreatitis or one that has features of chronic disease.[22,23] One challenge with the cerulein model is that responses between mice and rats are different. For example, the time course of zymogen activation, the degree of inflammation, and the pattern of cell death are distinct. Furthermore, the ability of repeated doses of cerulein to cause fibrosis in a chronic pancreatitis model depends on the mouse strain.[24] We believe that the cerulein model likely is relevant to initiator mechanisms in acute clinical pancreatitis, at least those that follow the earliest factors such as acinar cell–receptor activation. It also seems probable that prophylactic or therapeutic interventions that fail to show benefit in this model would not reduce injury in more severe models of acute pancreatitis. It is also the model best suited to identify or refute potential treatment targets in a cell biological context because it best preserves acinar cell physiology throughout the experimental disease course.[18,25]

### *Alcoholic Pancreatitis*

Although a number of models have used different avenues of delivery to generate alcoholic pancreatitis in rodents, the oral route given by diet or gastric infusion (and

probably the former) probably best reflects human exposure. A striking feature of these models is that animals do not develop pancreatitis.[26] Indeed, chronic alcohol feeding alone induces features of the endoplasmic reticulum stress response that protect against injury.[27] However, such ethanol exposure sensitizes rodents to the development of acute pancreatitis.[26] For example, rodents (rats and mice) chronically fed alcohol do not develop acute pancreatitis, but do so after co-treatment with physiological equivalents of cerulein.[26] Whether the down-regulation of the cystic fibrosis transmembrane conductance regulator by ethanol ingestion, shown in both rodents and human beings, is linked to this sensitization is unknown.[28] Potentially of greater clinical relevance, chronic ethanol feeding sensitizes rats to lipopolysaccharide (LPS), a bacterial product that is a potent stimulant of innate immune responses that signals through Toll-like receptor 4.[29,30] In this model of chronic alcohol ingestion in rats, LPS induces an acute pancreatitis response that persists and leads to chronic disease. However, continued exposure to alcohol is required to maintain the chronic pancreatitis response.[31] The ethanol-feeding LPS model may be one of the most relevant clinically. Increased serum LPS levels are found in human beings with chronic alcoholism, thus potentially setting the stage for alcoholic pancreatitis to develop.[32] We conclude that the failure for ethanol ingestion alone to induce acute pancreatitis reflects the human condition, and an additional sensitizing factor, such as LPS, or a genetic predisposition is needed to cause disease in animal models.

## Cigarette Smoke–Induced Disease

In the past decade, cigarette smoking has emerged as a risk factor for both acute and chronic pancreatitis.[33–35] Epidemiologic studies have suggested that its mechanism is different from alcohol. Animal models of pancreatic cigarette smoke toxicity are limited (reviewed by Alexandre et al[36]). Administration of liquid extracts of cigarette tobacco can cause mild pancreatic injury. Several studies have used nicotine or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a metabolite of nicotine that appears to mediate some of the toxicity of cigarette smoke.[37] NNK also has been found in pancreatic juice in concentrations up to 3 $\mu$mol/L. Given alone in vivo, nicotine and NNK cause mild pancreatic injury in short- and long-term studies. NNK is a potent agonist of several cell-surface receptors, including nicotinic receptors. In that context, using chemical inhibitors and mice with genetic deletions, several studies have found that pancreatitis effects of NNK (with acinar cells and in vivo) appear to be mediated by an $\alpha$-7-nicotinic receptor.[37] A very recent study suggested that one action of NNK in the pancreas was to reduce the activity of plasma-membrane and mitochondrial thiamine transporters.[38,39] This NNK effect was linked to reduced cellular mitochondrial function and adenosine triphosphate depletion. Although NNK is a clinically important component of cigarette smoke, it is likely that other constituents contribute to disease. Thus, studies using rodents exposed to chronic cigarette smoke are needed to determine whether it alone can cause pancreatic injury, affect thiamine transporters and mitochondrial function, or sensitize to other causes of acute pancreatitis such as ethanol ingestion and/or LPS. Whether cigarette smoke alone can cause acute pancreatitis or act as a sensitizing factor to other insults, such as alcohol or LPS, are important but open questions.

## Biliary Pancreatitis

It has been established firmly that the presence of gallstones is a significant etiologic factor for developing acute (but rarely chronic) pancreatitis, although the risk for gallstone carriers as a whole is minimal. It also has emerged that only gallstones small enough to migrate from the gallbladder into the duodenum can trigger disease onset.[40] The underlying mechanism, however, has been much debated. In 1901, Opie[41] postulated that impairment of the pancreatic outflow caused by gallstone obstruction of the pancreatic duct causes pancreatitis. This initial duct obstruction hypothesis was somewhat forgotten when Opie[42] published his second common channel hypothesis during the same year, which predicted that an impacted gallstone at the papilla could create a communication between the pancreatic and the bile duct (the so-called *common channel*), through which bile would flow into the pancreatic duct and would cause pancreatitis. This latter hypothesis received much attention because, during surgery in the late course of the disease, bile-stained pancreatic necrosis often was observed. Based on these observations, various models of biliary pancreatitis have been developed, including one that uses low concentrations of glycodeoxycholic acid infusion into the pancreatic duct, which causes moderate to severe pancreatitis in the rat when combined with cerulein.[43] This proposed disease mechanism remained popular despite a number of clinical inconsistencies such as the fact that in most human beings the communication between the pancreatic duct and the common bile duct is much too short (<6 mm) to permit biliary reflux into the pancreatic duct, and that an impacted gallstone most likely would obstruct both the common bile duct and the pancreatic duct.[44,45] Even in the event of an existing anatomic communication, pancreatic secretory pressure still would exceed biliary pressure and pancreatic juice would flow into the bile duct rather than bile into the pancreatic duct.[46,47] In the context of this question, an animal model has helped greatly in elucidating the underlying disease mechanism. The American opossum (*Didelphis virginiana*) has a biliary–pancreatic anatomy that resembles that in human beings, thus permitting the experimental conditions of bile duct obstruction, pancreatic duct obstruction, or simultaneous obstruction of both systems and reflux of bile into the pancreatic duct. In experiments using this opossum model it became clear that acinar cells are the initial site of injury during pancreatitis[48] and the impairment of flow from the pancreatic duct, rather than reflux of bile, triggers disease onset.[49] This insight later was confirmed by clinical studies involving human gallstone disease and pancreatitis[50–52] and now widely is accepted as the mechanism of biliary

pancreatitis.[53] To address the underlying cell biological mechanisms of how duct obstruction triggers pancreatitis, investigators were restricted to studying intracellular organelle trafficking,[54] and then resorted to rodent models to investigate relevant signaling events.[55] This choice was made for the simple reason that opossum studies inherently are limited in scope owing to a lack of information on protein structure, availability of antibodies, knowledge of genetic background, and nonavailability of inbred strains. The opossum model thus is an extreme example of how suitable an experimental model can be to address one narrow question regarding the pathogenesis of pancreatitis and our current limitations for studying other aspects of the disease. In this context, infusion of bile into the pancreatic duct under pressure causes severe pancreatitis in animal models that is limited to the pancreatic head.[56] Although this model meets the requirement of causing severe experimental disease, it probably does not reproduce the conditions that initiate human biliary pancreatitis.

## Obesity and Pancreatitis

A subject in which close similarities between human and animal models may exist is related to the worsening effects of obesity, especially intrapancreatic fat necrosis, on disease severity.[57] Both diet-induced obesity and congenital forms of obesity are used to model the human state. Because the congenital models often also show changes in leptin metabolism, a factor that can influence inflammatory responses, their relevance to human disease often is unclear because obese human beings do not have this defect. It seems likely that injurious factors released by both intrapancreatic fat and peritoneal fat stores can worsen pancreatitis injury.

The importance of fat is reflected by pancreatic pathology: one of the earliest signs of histologic damage in pancreatitis is fat necrosis. Although obesity, especially the accumulation of fat in the abdomen, directly corresponds to disease severity, fat located within the pancreatic parenchyma appears to be especially deleterious. In an elegant study that used both human materials and rodent models, a correlation between the levels of pancreatic fat, extent of fat necrosis, and disease severity were shown.[58] Based on studies performed in cellular models and using information derived from necrotic human tissues, a model of damaging fatty acid signaling between acinar and fat cells was proposed.[58,59] The length of free fatty acids as well as the degree of fatty acid saturation also may affect injury. Whether therapies aimed at reducing triglyceride hydrolysis by lipase to free fatty acids will be a useful therapy for clinical pancreatitis remains to be seen.

## Nutrient-Induced Pancreatitis: Choline-deficient Ethionine Supplemented Diet and Arginine or Other Basic Amino Acids

Although the mechanism remains unclear, administering excess basic amino acids or derivatives of naturally occurring amino acids can cause severe acute and sometimes lethal pancreatitis.[60] The mechanisms whereby these agents cause pancreatitis is unclear. Furthermore, the choline-deficient ethionine supplemented diet model shows selectivity, only causing severe disease in young female mice.[61] Among its other disadvantages, it has been difficult to avoid rapid severe responses in mice and the cost and care required to administer the choline-deficient ethionine supplemented diet. Furthermore, these extreme diets also cause damage to other organs such as the liver, which secondarily may affect the pancreas. It is known that inherited disorders of branched-chain amino acid metabolism, as well as conditions of extreme or selective malnutrition, greatly can increase the risk of developing pancreatitis in human beings.[62] It therefore is likely that diet-based models of pancreatitis have some relevance for human disease.

## Post-ERCP Pancreatitis

Since the inception of this procedure, it has been recognized to cause acute pancreatitis in 2%–15% of patients. The risk of developing this complication is related to features of the procedure and patient characteristics such as difficult cannulations; multiple injections; and patients who are young, female, and have had previous pancreatitis. Rodent models involve securing a cannula in the pancreatic duct and introducing contrast under pressure.[63] Initial studies on this topic showed that standard formulations of contrast, all containing a synthetic iodinated molecule needed for radiographic visualization, caused pancreatitis in rats.[63] A buffered solution of similar osmolality and pH, but lacking the iodinated compound, did not cause pancreatitis.[63] Activation of transient receptor potential vanilloid 1 neuronal receptors, which are pressure, heat, ethanol, and pH sensitive, and linked to neurogenic inflammation and pain, appeared to mediate this response.[64,65] More recent studies have suggested that one effect of the iodinated compound relates to its ability to induce pathologic calcium signaling in both rodent and human tissues.[66] Whether this effect is relevant to its activation of TRPV1 channels has not been explored. In these studies, pancreatitis responses were mild and the severe responses observed in a subset of human subjects were not observed. Although this preparation likely will provide useful mechanistic insights, its inability to cause severe PEP is a limitation. To improve the model, one option would be to determine whether a sensitizer factor such as LPS could shift these models to a more severe phenotype. Interestingly, although data suggesting that the nonsteroidal anti-inflammatory drugs diclofenac and indomethacin reduce the incidence of PEP in human beings, their effectiveness has not been confirmed in these animal models.[67] However, because rectal administration of these nonsteroidal anti-inflammatory drugs may be needed for effectiveness in human beings, it may be difficult to parallel such studies in animal models. The lack of a severe pancreatitis responses in current models also may limit the utility of these models.

## Drug-Induced Pancreatitis

Another perceived shortcoming is the difficulty of studying drug-induced pancreatitis in animal models to gain insight into the underlying mechanisms of onset. This was true for the early phase of the acquired immune deficiency syndrome epidemic when drug-induced pancreatitis was

common and animal studies were found to be unhelpful.[68] Similarly, the latest wave of anxiety about incretin-based antidiabetic agents has not been put to rest, although the evidence for pancreatitis responses in models has been almost entirely negative.[69,70] Differences between rodents and human beings in terms of genetic background, inherent metabolism, and possibly microbiota may account for the limitations of animal models to identify underlying disease processes or characterize susceptibility factors, especially when idiosyncratic drug reactions are concerned.

## General Mechanisms, Values, and Limitations of Models and Study Design

We are confident that the general pathobiologic responses characterizing acute pancreatitis, including the early activation of digestive enzymes, release of inflammatory mediators, enhanced paracellular permeability in vascular and epithelial tissues, inflammation, pancreatic cell death by apoptosis and necroptosis, and lung damage are shared in rodents and human beings. However, beyond superficial and select measurements, we often lack the data needed to state how similar the responses are—in part this is owing to the cost of performing careful measurements of such markers over time, and in the case of human material, the lack of access to tissue, with the exception of the rare samples obtained surgically (Table 1). Furthermore, most available tissue from human beings is collected, if at all these days, well after the early stages of the disease and not during disease initiation. In addition, the complexity of the disease in rodent models, the paucity of detailed data from human beings, and the infrequency with which beneficial therapeutic interventions have come from studies in pancreatitis models contribute to our lack of critical knowledge as to their relevance in a preclinical setting. Although significant progress has been made in identifying and characterizing the underlying pathophysiology and cell biology of pancreatitis using experimental models, to what extent the results in animal pancreatitis can predict the outcome of clinical trials that target the same disease mechanism remains an unanswered question. For example, we anticipate that disordering of cytosolic calcium signaling will be highly conserved as an early pathologic response in the pancreatic acinar cell, but the upstream pathways that induce this response could differ between model animals and human beings.[71] It also is likely that for an individual patient, multiple factors contribute to the risk of developing acute pancreatitis and/or modulate its severity. For example, epidemiologic studies show that the majority of alcohol abusers, smokers, and even individuals with common bile duct stones do not develop acute pancreatitis. Thus, differences in environmental factors and genetic make-up must influence which individuals with these risk factors will develop pancreatitis, but most probably remain unknown. Genetic studies tell us that a range of factors, such as those that are related to digestive enzyme metabolism in the acinar cell,[72–76] can impact our risk for pancreatitis. It is likely that future studies will describe other genetic factors that affect the risk of developing pancreatitis or modify its severity. However, it seems likely, if not certain, that most of these are obscured in the animal models we use to explore human disease unless the specific genetic alterations are introduced in the respective animal strain, a task now achieved more easily by the Clustered regularly interspaced short palindromic repeats (CRISPR) Cas-9 gene editing technology. Moreover, the genetic variations and environmental differences that affect human susceptibility are eliminated by the deep inbreeding and maintenance of strict environmental conditions characteristic of previous rodent models.

A shortcoming of experimental design is that most are short term and do not follow up animals to late complications or examine tissue reconstitution, with the notable exception of some chronic models.[21] An example of the translational importance of the latter is highlighted by the recent recognition that pancreatic islet $\beta$-cell failure may be seen as a late complication in 20%–30% of acute pancreatitis.[77] Differences in the microbiota, metabolism, diet, and regulation of inflammation can be substantial between human beings and rodents and affect disease mechanisms and phenotypes. Thus, a range of factors, including inherited genetic differences among species and study design, can influence the value of animal models. One example is the significant role that Toll-like receptor 4 has in regulating the severity in an animal model of pancreatitis, whereas in human disease loss-of-function polymorphism appears not to have such an effect.[78,79] Another example is the emerging recognition from models that trypsin activation is not required for the development of acinar cell necrosis,[80–82] whereas trypsin mutations (most probably associated with enhanced or a gain of function) are clearly the dominant genetic risk factors for pancreatitis in human beings.[13,83–85] It is likely that no model of pancreatitis will ever recapitulate all aspects of human disease, but individual aspects and mechanisms can be addressed specifically.

Another important issue is the timing of interventions with respect to disease onset. Prophylactic therapies are initiated before disease onset; therapeutic interventions are given after disease onset. Post-ERCP pancreatitis occurs in 2%–15% of individuals undergoing this procedure and represents one of the very few clinical scenarios in which one can administer prophylactic therapies.[86] Patients with other forms of pancreatitis often do not present for treatment until 24 hours after the onset of symptoms. Thus, opportunities to block early factors in acute pancreatitis may have passed by the time a patient arrives at the emergency department. Challenges in designing rational interventions in animal models include limited knowledge of the time course of disease and major mechanisms in human beings and rodents. For example, the appropriate time to use an agent that reduces neutrophil activation or modulates macrophage phenotype in an animal model as well as which parameters are the best measures of success remain unclear.[87–89]

It is widely held that injury to the pancreatic acinar cell is a key initiating step in acute pancreatitis. Over the past 2 decades, treatment of acinar cells with supraphysiologic concentrations of the cholecystokinin orthologue, cerulein, or cholinergic agonists such as carbamoyl choline, and bile salts have been used to induce injury in acinar cells.[90–92]

Pancreatitis responses in these cells include changes in calcium signaling with a loss of physiologic oscillations, a high spike in cytosolic calcium, followed by a slow return to basal levels.[71,93–95] Potential interventions that affect acinar cell calcium signaling are discussed later. Changes in other signaling pathways, such as select protein kinase C isoforms, and activation of nuclear factor-$\kappa$B, also are early acinar cell pancreatitis responses.[96–98] In rodent acinar cells, these soon are followed by intracellular activation of digestive zymogens such as trypsinogen,[99] stimulation of and elaboration of inflammatory mediators, membrane and cytoskeletal disruption,[100–102] decreased mitochondrial function and oxidative stress,[93,103,104] decreased autophagic flux,[105,106] reduced secretion,[92,107] and activation of cell-death pathways. Recent access to freshly isolated human tissue from surgical procedures, as byproducts of preparing pancreatic islets for transplantation, or archival fixed material, has shown that at least some of the rodent acinar cell pancreatitis responses are conserved. This includes disordered autophagy and calcium signaling.[16,108] Although much of the work in this area has appeared only in abstract form or is in progress, the preliminary studies have suggested that key human pancreatitis responses in the pancreatic acinar cell will be conserved in rodent acinar cells.

## Has Anything Been Identified in Animal Models That Clearly Is Applicable to Human Disease?

The most important reason to study animal models of acute pancreatitis is the hope that we can improve the natural history of disease in human beings. This could take advantage of opportunities to provide prophylactic therapy that blocks the onset of acute pancreatitis or therapeutic interventions that are given after disease onset to reduce its severity. However, there may be only a few examples in which the many studies in animal models have informed clinical studies and patient outcomes positively or in which a beneficial effect in experimental pancreatitis has translated into a beneficial effect in human beings.

Although intravenous fluids are considered a cornerstone of acute pancreatitis therapy, relatively little has been performed in animal models until recently to explore the effects of different types and amounts of intravenous buffers. Prompted by a preliminary study showing that lactated Ringer's solution appeared to reduce inflammatory responses compared with normal saline in a cohort of patients with severe acute pancreatitis,[109] several basic science studies in rodents have been performed. One study showed that a low-pH environment, such as might occur in the pancreatic parenchyma early in the course of acute pancreatitis, could worsen disease.[110] This finding could be relevant to the low pH of normal saline ($\sim$5.3), especially when it is given in large volumes. Subsequent studies have shown that lactate, acts on a G-protein receptor (G-protein receptor 81) to suppress critical innate immune responses in acute pancreatitis and lessens disease severity.[111] We anticipate that emerging studies will examine the effects of administering other metabolic intermediates on acinar cell and inflammatory responses in acute pancreatitis and that clinical studies will optimize the rational use of therapies that modulate a range of acute inflammatory responses in this disease. In this case (modulation on innate immune responses), the available studies have suggested that animal models may reflect human responses and inform interventions.[112] The effects of differing volumes of intravenous fluids also needs to be studied.

The central role of abnormal calcium signaling in the acinar cell, including a demonstration of such abnormalities in an animal model of biliary pancreatitis, have made this an attractive therapeutic target.[55] Several recent approaches to reducing pathologic acinar cell calcium signaling hold promise for human therapy. First, increasing extracellular magnesium reduces pancreatitis responses in rodent acinar cells including pathologic calcium signaling and lessens the severity of in vivo pancreatitis in rodent models[113,114]; it now is being examined for its potential role in reducing PEP.[112] Insulin recently was shown to decrease calcium levels caused by induction of pancreatitis responses in acinar cells and reduce other cellular pancreatitis injuries.[115,116] These actions appear to be linked to a plasma-membrane calcium pump. Whether such protective effects of insulin can be shown in in vivo preparations awaits study. More recently, drugs have been developed that reduce calcium entry into the acinar cell and also dramatically reduce pancreatitis responses in isolated human acini.[16] Because abnormal acinar cell calcium signaling is largely an early response, such interventions might be most useful to prevent PEP. However, the efficacy of these agents may extend to the inflammatory response and provide a rationale for their use in clinical pancreatitis.

One example of a medication that generally is accepted as beneficial in human beings (PEP) but has relatively little data in rodent models to support its use in human beings is indomethacin and related anti-inflammatory drugs. Almost 40 years ago, indomethacin was found to be a potent inhibitor of $Ca^{2+}$-activated polymorphonuclear phospholipase A2 (PLA2).[117] Soon after, PLA2 levels were found to be increased in the serum of subjects with acute pancreatitis and the degree of the increase corresponded to severity.[118] Roles from PLA2 from inflammatory as well as acinar cells in the pathogenesis of acute pancreatitis were proposed. More than 100 research publications and reviews on the topic followed with emphasis on its role in renal and lung injury.[119] Differences were observed with the prophylactic administration of PLA2 inhibitors in severe pancreatitis models.[120,121] However, indomethacin showed little benefit in a rat model of acute pancreatitis compared with a selective Cox2 inhibitor. Indomethacin transiently, but prominently, reduced pancreatic blood flow in pigs and also has been found to reduce perfusion in other vascular beds.[122] To our knowledge, no tests of the relative effectiveness of different administration routes for indomethacin were studied before the initiation of clinical trials. Nonetheless, several, but certainly not all, clinical studies of indomethacin have suggested a benefit in reducing the severity of post-ERCP pancreatitis.[67,123] Although this appears to be one of the few successful drug interventions

for this disease, it would be difficult to argue that the data from experimental pancreatitis models predicts this benefit. Thus, with relatively little experimental support, indeed with some negative studies, clinical investigators could show unpredicted treatment benefit in human beings.

## Conclusions

The issue of whether an acute pancreatitis model reflects human disease should be evaluated in the context of the issue being addressed. Thus, it is important to consider the goal of the studies, especially if it is to develop a therapeutic intervention that could improve the natural history of a disease. This can be performed prophylactically, such as interventions to prevent post-ERCP pancreatitis, and therapeutically, when the disease already is established and corresponds to the clinical conditions and practice. It can be argued that if critical pathways are shared among the common causes of acute pancreatitis and between human beings and animal models, that agents that are effective in these settings would have value irrespective of the initiating factor. Thus, the precise pathways that initiate alcoholic and biliary pancreatitis are of considerable scientific interest, but if early and late mechanisms are shared by different etiologies of acute pancreatitis, it may not be critical to mimic the precise conditions that initiate disease in human beings. Our information related to factors that initiate and perpetuate acute pancreatitis still is being investigated. Additional time and research is needed to produce the data needed to make highly informed comparisons between animal models and human beings. Furthermore, a major challenge is the difficulty in performing clinical trials to confirm the value of potential therapies identified in animal models. Although acute pancreatitis is not an uncommon disease,[124] its course is extremely variable and very difficult to predict, thus necessitating large study cohorts. Furthermore, the factors that provide the prognostic information needed to efficiently conduct clinical trials still are evolving. In this context, model investigative units for carefully designed collaborative studies provide examples of how the field might best move forward. Animal models have contributed greatly and successfully to the elucidation of some of the fundamental disease mechanisms of pancreatitis and will continue to do so. Before they can be translated consistently into improved care and better outcomes for pancreatitis patients, the field needs time, better funding, and a greater focus on patient-relevant results.

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