

LEVAN BENASHVILI<sup>1</sup>, DAVIT TOPURIA<sup>2</sup>, MAIA MATOSHVILI<sup>3</sup>, INGA KAKHNIASHVILI<sup>4</sup>

## MODELS OF CHRONIC PANCREATITIS

Tbilisi State Medical University, departments: <sup>1</sup>Clinical Anatomy and Operative Surgery; <sup>2</sup>Human Normal Anatomy, <sup>3</sup>Dermatology and Venereology, <sup>4</sup>Clinical Skills

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ლევან ბენაშვილი<sup>1</sup>, დავით თოფურია<sup>2</sup>, მაია მათოშვილი<sup>3</sup>, ინგა კახნიაშვილი<sup>4</sup>

### ქრონიკული პანკრეატიტის მოდელები

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, დეპარტამენტები: <sup>1</sup>კლინიკური ანატომიისა და ოპერაციული ქირურგიის; <sup>2</sup>ადამიანის ნორმალური ანატომიის; <sup>3</sup>დერმატოლოგიისა და ვენეროლოგიის; <sup>4</sup>კლინიკური უნარების

### რეზიუმე

ცხოველებში მწვავე და ქრონიკული პანკრეატიტის ლაბორატორიული მოდელები იქმნება პათოგენეზის მექანიზმების შესასწავლად, პანკრეასის ანთების დროს მკურნალობისა და კანცეროგენეზის ახალი მეთოდების შემუშავების მიზნით. ორივე ინ ვიტრო მოდელი გამოიყენება ადრეული სტადიის, მოკლევადიანი პროცესების შესასწავლად, რომელშიც ჩართულია აცინარული უჯრედები, ასევე მოდელები, რომლებიც იწვევენ მღრღნელებში დაავადების მსუბუქი ან მძიმე ფორმების განვითარებას.

მიუხედავად იმისა, რომ მღრღნელები ყველაზე ხშირად გამოიყენება პანკრეატიტის მოდელებში, მათ მიერ გამოწვეული პანკრეასის დაზიანება სულაც არ შეესაბამება ადამიანის პათოლოგიას. აქედან გამომდინარე, აუცილებელია ფრთხილად აირჩიოთ მოდელი, რომელიც ყველაზე შესაფერისია ამ კონკრეტულ ამოცანაში პასუხისთვის. ამ სტატიის მიზანია პანკრეატიტის ყველაზე გავრცელებული ცხოველური მოდელების შედარება.

**Models of chronic pancreatitis.** Two general mechanisms for the development of chronic pancreatitis in humans have been proposed. In the first case, numerous subclinical or clinically pronounced attacks of acute pancreatitis lead to chronic pancreatitis. In the second case, one initial and often severe injury creates conditions that perpetuate and lead to chronic disease without the need for repeated severe injury. Animal model studies indicate that both mechanisms can lead to chronic pancreatitis. As in the case of acute pancreatitis, few models of chronic pancreatitis use injury mechanisms that are likely to be associated with the pathogenesis of human diseases, and most others cause disease through mechanisms that have no clear clinical relevance. Since the final common pathways of disease progression, models can be used to study therapeutic agents even if the mechanism initiating the disease is not of clinical relevance. Few, if any, models demonstrate all the features of human disease, which include the loss of exocrine and endocrine cells. Regarding models of acute pancreatitis, the choice of model should be based on experimental question and knowledge of the human pancreatic response.

**Mechanical Models.** Partial, selective or complete obstruction of the pancreatic duct is often used to produce chronic damage to the pancreas. The progression of the disease depends on the species; rats develop fibrosis faster than dogs. Anatomical differences between species also influence the development of the disease. For example, rats have a single main outflow tract that easily becomes obstructed, causing damage to the entire gland. However, the mouse pancreas has several pancreatic ducts that differ significantly - this feature makes complete obstruction of the pancreatic ducts difficult, but at the same time provides internal control, since it is easy to obstruct only a subset of pancreatic segments. Obstruction of only one duct in rats practically does not lead to pancreatitis, but in combination with mild stimulation with secretogens causes acute pancreatitis [1-3]. Such stimulation can be used to simulate chronic pancreatitis [4]. Combined chronic ethanol feeding and partial obstruction of the pancreatic duct in dogs also causes chronic pancreatitis similar to that seen in humans. However, the high cost is one of the reasons why the model is rarely used [5].

**Biological, chemical and environmental factors.** Repeated injections of caerulein in mice and rats for several weeks cause chronic pancreatitis, which develops after repeated attacks of acute pancreatitis [6]. Caerulein also causes collagen deposition and pancreatic fibrosis, but these signs regress after injections are stopped. TGF-1 expression is believed to mediate the response, possibly by activating cells that promote fibrosis and influencing acinar cell regeneration. This model has become an important tool to study the influence of inflammation on the development of pancreatic neoplasia [7], genes mediating pancreatic repair after the origin of pancreatic stellate cells that mediate fibrosis [8,9]. Neither short-term administration of cerulein, nor short-term administration of cerulein (1 day), nor long-term intraperitoneal administration of ethanol cause signs of chronic pancreatitis. However, the combination of these substances leads to pronounced fibrosis of the pancreas, activates stellate cells and causes inflammation of the pancreas [10]. Although not fully characterized, this mouse model has the advantage of inducing transient pancreatitis. The model has the advantage of producing transient and alcohol-induced effects without the need for feeding. Whether damage to the pancreas persists, even after alcohol is stopped, remains to be seen. A well-characterized model that has a mechanistic relationship to human disease has been established in rats using a combination of chronic ethanol feeding and administration of a bacterial cell wall component, lipopolysaccharide (LPS) [11]. LPS is believed to contribute to pancreatic injury by activating innate immune pathways that have been associated with the pathogenesis of acute pancreatitis in humans (eg, pathways involving Toll-like receptor 4 and CD14. In addition, serum LPS levels are elevated in people who abuse alcohol. Sprague-Dawley rats fed an alcohol-enriched LieberDeCarli diet for 10 weeks and then injected with LPS developed acute acinar cell damage. LPS, acute acinar cell damage, stellate cell activation, and fibrosis developed [11]. A follow-up study showed that with continued alcohol intake, stellate cell activation and fibrosis persisted, but resolved shortly after alcohol was stopped. The LPS/alcohol model is attractive because of its potential clinical relevance and relative simplicity for rat studies. However, it is very difficult to feed alcohol to mice, so intraperitoneal administration of alcohol should be considered as an alternative [10]. Further research is needed to determine whether LPS and alcohol treated rats develop other features of chronic pancreatitis such as exocrine cell mass loss and pancreatic insufficiency, pain, sensitization, intraductal plugging, and loss of exocrine tissue. A convenient model of chronic pancreatitis that is also toxic to the hepatobiliary system has been identified in toxicological screening studies and is created by a single intravenous injection of dibutyltin dichloride (DBTC; 8 mg/kg/body weight) in rats [13]. DBTC causes acute inflammation of the pancreas within 24 hours, which progresses to chronic inflammation after a week, and then to a progressive fibrotic lesion over the next 2 months, with chronic and acute inflammation (mediated by T cells and macrophages). Levels of TGF-1 in the pancreas, a likely mediator of pancreatic fibrosis, correspond to collagen levels by collagen expression. This model has been used to study pain response 6 days after pancreatitis induction, and studies of this model have identified interleukin (IL)-6 and bradykinin receptors as a pain mediator [14,15]. In this model, the protease inhibitor camostat mesylate and taurine reduced fibrosis. The induction of pancreatitis in rats by DBTC may be of clinical importance because the compound was reported to cause pancreatic damage in East German shipbuilders when it was used as a paint component in Germany. A variation in the pattern of duct obstruction may be caused by the administration of trinitrobenzenesulfonic acid into the main duct of the rat pancreas [16]. Trinitrobenzenesulfonic acid, entering the large intestine, also causes colitis. Its administration into the pancreatic duct causes progressive fibrotic damage, duct stricture, glandular atrophy, and acute and chronic inflammation in a large number of rats. This model has been used to study pain caused by pancreatitis [17]. As previously described, the CDE diet causes a severe and usually fatal form of acute pancreatitis, especially in young female mice. However, mice fed an intermittent CDE diet (3 days of CDE diet alternated with 3 days of normal diet) developed histological signs of chronic pancreatitis over a long period of time (24 weeks) [18]. However, other features of the disease or the reversibility of lesions have not been studied. Although this model had few technical problems, the long-term CDE diet required for the development of the disease is costly and requires careful control of food intake.

**Genetic models.** Genetic manipulations have been used to create several models of chronic pancreatitis. Genetic changes in all tissues, and especially in ductal or acinar cells, can cause chronic pancreatitis in animals. Some of these genetic changes are homologous to those associated with human disease, such as in the genes encoding the cystic fibrosis transmembrane conductance regulator (CFTR) and KRAS. However, the impact of genetic variants can be complex and vary between species. For example, impaired CFTR in mice causes mild pancreatitis at best or exacerbates the severity of pancreatitis induced by other methods [19]. However, impaired CFTR in pigs causes the same rapidly progressive pancreatic disease that develops in patients with cystic fibrosis [20,21]. IL-1 is an inflammatory cytokine and mediator of acute pancreatitis that is overexpressed in some models of chronic pancreatitis. Expression of IL-1 under the control of the elastase promoter in the pancreas of mice induces pronounced histological signs of chronic pancreatitis and a T cell-dominated inflammatory response [22]. However, these mice do not develop acute pancreatitis. Although pancreatic fibrosis is more pronounced when mice are 20 weeks of age than in mice with caerulein-induced chronic pancreatitis, IL-1 transgenic mice develop neither pancreatic exocrine nor endocrine insufficiency after 8-10 months of age. Transgenic expression of activated Kras in mouse acinar cells results in histological features of chronic pancreatitis, stellate cell activation, and chronic pancreatic inflammation [23]. This model may be of great clinical and mechanistic importance, since Kras-activating mutations are observed in approximately 30% of patients with chronic pancreatitis and are the most common mutations found in pancreatic adenocarcinomas (90%). It has recently been reported that activated Kras induces a prolonged chronic inflammatory response in the mouse pancreas, which requires activation of nuclear factor- $\kappa$ B and subsequent activation of cyclooxygenase-2 [24]. Similar observations have been made in zebrafish expressing the oncogenic KRAS in the pancreas [25].

**Immune pancreatitis.** Immune-associated pancreatitis models develop the disease in a pattern that usually follows the human autoimmune pancreatitis (AIP) model. Type I AIP is associated with a periductular lymphoplasmacytic infiltrate, while type II is characterized by a predominantly neutrophilic infiltrate that may involve the ductal epithelium [26]. AIP type II, but not type I, is often associated with inflammatory bowel disease. MRL/Mp mice develop a form of autoimmune pancreatitis, 90 more frequently and earlier in females. The administration of polyinosinic:polycytidylic acid to these mice significantly shortens the course and increases the incidence of pancreatitis and biliary tract lesions. Interestingly, IL-10  $-/-$  mice, a widely used model of inflammatory bowel disease, developed type I AIP rather than the expected type II associated with human colitis [27]. In other AIP models, disease is induced by immunization with lactoferrin, other antigens, or alteration of commensal bacteria [28,29]. These models have not yet found wide application.

**Conclusions.** The risk of developing pancreatitis and the severity of acute or chronic disease are determined by a combination of genetic and environmental factors. For example, in patients who combine alcohol intake and cigarette smoking, smoking appears to increase the risk of developing acute and chronic pancreatitis. The effects of alcohol and smoking are independent and have different mechanisms of action. We have considered several models that take advantage of the synergy between different types of damage for the occurrence of disease. These models are especially useful to study when they have related or overlapping mechanisms of pathogenesis. For example, in rodent models, neither chronic alcohol consumption nor physiological concentrations of caerulein cause acute pancreatitis, but do when taken together. Similarly, neither alcohol nor LPS by themselves cause chronic pancreatitis in rats - the disease develops only when they are combined. In addition, obesity also appears to increase the severity of acute pancreatitis in humans [30] (especially when combined with intrapancreatic fat) and increase rodent susceptibility to cerulein- and cytokine-induced disease. Knowledge of the pathogenesis of human diseases should allow future researchers to develop models of pancreatitis that combine the relevant factors and therefore better reflect the characteristics of the disease observed in patients.

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*ЛЕВАН БЕНАШВИЛИ<sup>1</sup>, ДАВИД ТОПУРИЯ<sup>2</sup>, МАЙА МАТОШВИЛИ<sup>3</sup>, ИНГА КАХНИАШВИЛИ<sup>4</sup>*  
**МОДЕЛИ ХРОНИЧЕСКОГО ПАНКРЕАТИТА**

Тбилисский Государственный Медицинский Университет, департаменты: <sup>1</sup>Клинической Анатомии и Оперативной Хирургии, <sup>2</sup>Нормальной Анатомии Человека, <sup>3</sup>Дерматологии и Венерологии, <sup>4</sup>Клинических Навыков

**РЕЗЮМЕ**

Лабораторные модели острого и хронического панкреатита у животных создаются с целью изучения механизмов патогенеза, разработки новых методов лечения и канцерогенеза при воспалении поджелудочной железы. Используются как модели *in vitro* для изучения ранних стадии, краткосрочных процессов, в которых участвуют ацинарные клетки, так и модели, вызывающие развитие легкой или тяжелой формы заболевания у грызунов.

Хотя грызуны наиболее часто используются в моделях панкреатита, поражение поджелудочной железы, вызываемое у них, не обязательно полностью соответствует человеческой патологии. Поэтому необходимо тщательно выбирать модель, наиболее подходящую для ответа в данной конкретно поставленной задаче. Целью данной статьи является сравнение наиболее широко используемых моделей панкреатита на животных.

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**MODELS OF CHRONIC PANCREATITIS**

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**SUMMARY**

Laboratory models of acute and chronic pancreatitis in animals are created in order to study the mechanisms of pathogenesis, develop new methods of treatment and carcinogenesis during inflammation of the pancreas. Both *in vitro* models are used to study the early stage, short-term processes in which acinar cells are involved, as well as models that cause the development of mild or severe forms of the disease in rodents.

Although rodents are most commonly used in models of pancreatitis, the pancreatic damage they cause does not necessarily fully correspond to human pathology. Therefore, it is necessary to carefully choose the model most suitable for the answer in this particular task. The purpose of this article is to compare the most widely used animal models of pancreatitis.

**Keywords:** Laboratory models, Pancreatitis, acute, chronic.