The utilization of animal models in investigation of the pathomechanism of acute pancreatitis

Summary of Ph.D. thesis

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Publications related to the subject of the thesis

I. **Kui B**, Balla Z, Végh ET, Pallagi P, Venglovecz V, Iványi B, Takács T, Hegyi P, Rakonczay Z Jr.: Recent Advances in the Investigation of Pancreatic Inflammation Induced by Large Doses of Basic Amino Acids in Rodents. *Lab Invest.* 2014;94:138-149. **IF: 3.676**

II. **Kui B**, Balla Z, Vasas B, Végh ET, Pallagi P, Kormányos ES, Venglovecz V, Iványi B, Takács T, Hegyi P, Rakonczay Z Jr: New Insights into the Methodology of L-Arginine-Induced Acute Pancreatitis. *PLoS ONE* 2015;10(2): e0117588. **IF: 3.057**

III. Maléth J, Balázs A, Balla Z, **Kui B,** Katona M, Judák L, Németh I, Pallagi P, Kemény LV, Rakonczay Z Jr, Venglovecz V, Földesi I, Pető Z, Somorácz Á, Borka K, Perdomo D, Lukacs G, Gray AM, Monterisi S, Zaccolo M, Sendler M, Mayerle J, Kühn J, Lerch MM, Sahin-Tóth M, Hegyi P: Alcohol Disrupts Levels and Function of the Cystic Fibrosis Transmembrane Conductance Regulator to Promote Development of Pancreatitis. *Gastroenterology* 2015; 148(2):427-439. **IF: 18.187**

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Publications not related to the subject of the thesis

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II. Lakatos G, Balázs A, **Kui B**, Gódi S, Szücs Á, Szentesi A, Szentkereszty Z, Szmola R, Kelemen D, Papp R, Vincze Á, Czimmer J, Pár G, Bajor J, Szabó I, Izbéki F, Halász A, Leindler L, Farkas G Jr, Takács T, Czakó L, Szepes Z, Hegyi P, Kahán Z.: Pancreatic Cancer: Multicenter Prospective Data Collection and Analysis by the Hungarian Pancreatic Study Group. *J Gastrointest Liver Dis*. 2016;25(2):219-25. **IF: 1.891 (2015)**

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List of abbreviations

AP: acute pancreatitis

CCK: cholecystokinin

CDE: choline-deficient, ethionine-supplemented

CFTR: cystic fibrosis transmembrane conductance regulator

ERCP: endoscopic retrograde cholangio-pancreatograpy

FA: fatty acid

FAEE: fatty acid ethyl ester

i.p.: intraperitoneal(ly)

IL: interleukin

KO: knock-out

MPO: myeloperoxidase

NHERF-1: Na^+/H^+ exchanger regulatory factor-1

PA: palmitic acid

POA: palmitoleic acid

PS: physiological saline

WT: wild-type

Introduction

The physiology of the pancreas

The pancreas consists of 82 % acinar cells, 8 % duct cells, 4 % blood vessels, 2 % endocrine cells and 8 % extracellular matrix. The function of acinar cells is to secrete digestive enzymes. The main stimulus of enzyme secretion is the vagal nerve stimulation, releasing acetylcholine, and the effects of circulating cholecystokinin. Both acetylcholine and CCK cause the elevation of intracellular Ca^{++} concentration in pancreatic acinar cells. Acinar cells also produce Cl^--rich isotonic fluid. Digestive enzymes which are produced by acinar cells are packed into zymogen granules and are secreted by exocytosis. In the gut lumen enterokinase converts trypsinogen to trypsin, then active trypsin activates other proenzymes to active enzymes.

The pancreatic ductal epithelium secretes 1.5-2 liters of alkaline fluid daily that may contain up to 140 mM $NaHCO_3$. The physiological function of this alkaline secretion is to wash digestive enzymes down the ductal tree and into the duodenum, and to neutralize the acid chyme entering the duodenum from the stomach. HCO_3^- is accumulated across the basolateral membrane of the duct cell by Na^+/HCO_3^- co-transporters and by the backward transport of protons via Na^+/H^+ exchangers (NHE) and H^+-ATPase. Pancreatic HCO_3^- secretion across the apical membrane of epithelial duct cells is thought to be mediated by anion channels and transporters such as cystic fibrosis transmembrane conductance regulator (CFTR) and SLC26 anion exchangers. CFTR is an ATP-gated anion-channel, which allows the flow of anions (Cl^-, HCO_3^-) according to their electrochemical gradients. Mutations of CFTR are known to cause cystic fibrosis, but less commonly they can also lead to pancreatitis.

Acute pancreatitis

Acute pancreatitis (AP) is one of the most challenging gastroenterological diseases which often requires hospital admission. The annual incidence rate is 10–45 cases per 100000 people in Western countries, however, it shows increasing tendency. In the United States, this disease accounts for 200,000 hospital admissions annually, at a cost of $ 2-2.5 billion.

The etiology of AP is considered to be multifactorial, although the different triggering mechanisms are thought to end up in the same common pathway. The most common causes of AP are massive alcohol consumption and cholelithiasis. Other causes include genetic

alterations, hyperlipidemia, hypercalcemia, drugs and toxins, infections, pancreatic tumors, pancreas divisum, autoimmune diseases and vascular disorders.

The pathogenesis of AP is not well understood, it has no specific treatment, therefore investigating the pathogenesis of acute necrotizing pancreatitis and searching for new therapeutic options is more than justified.

Animal models of experimental acute pancreatitis

Animal models are often used in research of AP. Each method has some advantages, but also some disadvantages. There are invasive and non-invasive AP models.

The most commonly investigated AP model is induced by repetitive injections of supramaximal doses of **secretagouges** (like CCK or its analogue cerulein). Cerulein treatment causes mild, edematous pancreatitis in rats and severe inflammation and cell damage in mice. Secretagouges inhibit digestive enzyme secretion, cause premature trypsinogen activation, vacuole formation, activation of transcriptional factors, increase of proinflammatory cytokine concentrations in the serum and decrease acinar protein synthesis.

The **retrograde injection of bile acid** is a very popular AP model, however this technique requires general anesthesia and surgical procedure. The model is based on the common channel theory (the main pancreatic Wirsung duct and the bile duct have a common part), which was described by Opie in 1901. In this model, the Wirsung duct is cannulated and bile acid is injected into the pancreatic duct. This model mimics the development of AP after endoscopic retrograde cholangiopancreatography (ERCP).

The choline **deficient, ethionine-supplemented** (**CDE) diet-induced AP model**, is not commonly used. It causes severe necrotizing pancreatitis with hemorrhage, but it works only in young female mice, and this diet is very expensive.

Basic amino acid-induced (L-arginine, L-ornithine, L-lysine) AP model is very popular in rats, and the use of this model in mice is also increasing nowadays. Basic amino acid-induced AP models only affect pancreatic acinar cells, pancreatic ductal and endocrine cells show no signs of damage. The advantages of using basic amino acid-induced AP models are relatively straightforward. These models are cheap, technically very simple to carry out and only require i.p. injection(s). The method of induction is non-invasive and does not require any anesthesia or surgery. They nicely reproduce most laboratory and morphological features of human AP. One important difference with respect to the morphological features of AP is that

the human disease is usually patchy in distribution, whereas basic-amino-acid-induced AP is relatively homogenous.

The **ethanol and fatty acid-induced AP** model is one of the most recently described forms of AP induction in rodents. This model is based on the fact that alcohol intake and consumption of a heavy meal can cause AP in humans. Interestingly, alcohol or fatty acid administration separately can't induce AP in rodents. Alcohol is metabolized via oxidative (alcohol dehydrogenase and microsomal enzyme system) and non-oxidative pathways (free fatty acids). The latter, predominant route generates fatty acid ethyl esters (FAEEs) from fatty acid substrates in the pancreas including carboxylester lipase, an enzyme synthesized and secreted by the acinar cells. Inhibition of the oxidative pathway promotes formation of FAEEs, which induce sustained elevations of cytosolic calcium concentration leading to inhibition of mitochondrial function, loss of ATP and necrosis of isolated pancreatic acinar cells.

Huang *et al.* described in 2014 that i.p. injections of ethanol (1.35 g/kg), in combination with palmitoleic acid (POA) (150 and 300 mg/kg), induced pancreatic damage 24 h after administration of POA and ethanol, with acinar cell damage, pancreatic interstitial oedema, neutrophil infiltration.

Aims

Characterization of L-arginine-induced AP in mice

The L-arginine-induced AP model in mice described by Dawra *et al*. (2007) wasn't working well in our hand, because we found either high mortality in C57Bl/6 mice or no mortality but only mild disease in Balb/c mouse strain. Therefore, we investigated the: dose -concentration -gender and strain -dependency of L-arginine-induced AP in mice.

The effects of CFTR inhibition on the severity of AP

We modified and used the experimental AP model of Huang *et al*. to investigate the role of pancreatic ductal CFTR in experimental AP by comparing the severity in CFTR knock-out (KO) mice and their wild-type (WT) littermates.

Materials and methods

All experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the University of Szeged and also by an independent committee assembled by national authorities. Male and female mice weighing 20–25 g were used. FVB/n, C57BL/6 and BALB/c mouse strains were from Charles Rivers Laboratory. Due to ethical issues, particularly in case of groups that needed euthanasia in large proportions, the laboratory and histological analyses were performed on a lower number of mice (in order to avoid the unnecessary death of animals).

CFTR KO mice were generated by Ratcliff *et al.* (1993) on an FVB/n background and were kind gifts of Prof. Ursula Seidler. The animals were allowed free access to specific CFTR chow and drinking solution to allow survival beyond weaning. All mice were genotyped by PCR analysis before the experiments. All chemicals were purchased from Sigma-Aldrich unless indicated otherwise.

L-arginine-HCl was dissolved in physiological saline (PS), and its pH was set to 7.4 with NaOH. Different concentrations (5, 8, 10 and 30 %) and doses (2×4, 3×3 and 4×2.5 g/kg, administered at hourly intervals) of L-arginine-HCl were administered intraperitoneally (i.p.). Control animals were treated i.p. with PS instead of L-arginine-HCl. Mice were sacrificed at the peak of pancreatic injury, 72 h after the first i.p. injection. Animals were exsanguinated through cardiac puncture, the pancreas was rapidly removed and trimmed from fat and lymph nodes on ice. Parts of the pancreatic tissue were immediately frozen in liquid nitrogen then stored at -80°C until biochemical assays were performed. Another part of the pancreas was fixed in 6% neutral formaldehyde solution for histological analysis. Blood samples were centrifuged at 2500 RCF for 15 min at 4°C and the serum was stored at -20°C until use. The mice became sluggish and lethargic soon after the i.p. L-arginine-HCl injections. Some mice recovered within 12 h after the injections, but others remained unwell. Based on preliminary experiments and literature data, if the core temperature measured rectally with a digital thermometer of the latter mice decreased to critical levels (27–29 °C) 12 h after the L-arginine injections, the animals were euthanized by pentobarbital overdose to minimize suffering. The postmortem macroscopic and histological analysis proved that these mice did not suffer from AP. The surviving mice gradually recovered from the L-arginine-HCl injections and either developed necrotizing AP or remained AP-free by 72 h (when the experiment was terminated). The effectivity rate (in %) of AP induction was defined as (the number of mice which showed

signs of AP divided by the total number of mice injected with L-arginine) × 100. The signs of AP on histological analysis included pancreatic edema, acinar cell damage, and inflammatory cell infiltration. The euthanized animals and those that did not show any signs of AP according to the histological analysis were excluded from the measurements (except in case of BALB/c mice treated with 2×4 g/kg 5 % L-arginine-HCl which recovered from the L-arginine-HCl injections and none of the animals demonstrated signs of AP).

We modified the original protocol of Huang *et al*. (2014) and applied one injection of the mixture of alcohol and fatty acid mixture, instead of two, since the mice in our hands tolerated this treatment better. Wild-type (WT) and CFTR KO mice were treated with a mixture of 1.75 g/kg ethanol and 750 mg/kg palmitic acid (PA) *i.p.* Before the ethanol and PA treatment, mice were injected with 200 µl PS to avoid ethanol-induced peritoneal irritation. The control mice were treated with 200 µl PS *i.p.* instead of the mixture of ethanol and PA. Using the modified protocol, no mortality was observed. Mice were sacrificed 24 h after the ethanol and PA or PS treatment by exsanguination through the heart under pentobarbital (85 mg/kg *i.p.*) anaesthesia.

Serum amylase activity was measured with a colorimetric kinetic method using a commercial kit. To evaluate the water content of the pancreas, the pancreatic wet weight was measured, then the tissue was dried for 24 h at 100 °C and the dry weight was also measured. The dry weight (DW) and wet weight (WW) ratio was calculated as: (1-DW/WW)×100. Pancreatic myeloperoxidase (MPO) activity is a hallmark of leukocytic infiltration and was measured according to Kuebler *et al*. (1996). MPO activities were normalized to total protein content measured by the Lowry method. To determine the extent of inflammatory response in the pancreata, we measured interleukin (IL)-1β levels using a commercial ELISA kit as described previously. Tissue samples were stained with hematoxylin and eosin. Pancreatic sections were analyzed and scored by pathologists blinded to the experimental protocol. 6-8 fields were checked and scored at 100× magnification. Edema was scored from 0–3 points (0: none; 1: patchy interlobular; 2: diffuse interlobular; 3: diffuse interlobular and intraacinar), leukocytic infiltration from 0–3 points (0: none; 1: patchy interlobular; 2: diffuse interlobular; 3: diffuse interlobular and intraacinar), the percentage of acinar cell necrosis was evaluated by ImageJ software. Normal, non-AP pancreatic samples from L-arginine-treated mice were eventually excluded from the data analysis unless indicated otherwise.

Data were presented as means ± SEM. Differences in euthanasia rates (mortality) were determined by chi square test with Yates correction. Laboratory and histological parameters were evaluated by using the two–way analysis of variance (ANOVA) followed by Bonferroni

post hoc test, if the distribution of data was normal. Kruskal-Wallis non-parametric test with Dunnett's multiple comparison post hoc test was used, if the distribution of data was not normal. $P<0.05$ was accepted as statistically significant.

Results

The effects of L-arginine concentration on the development of acute pancreatitis in BALB/c, C57BL/6 and FVB/n mice

In the original article by Dawra *et al*. (2007), the 2×4 g/kg L-arginine-HCl dose was administered as an 8 % L-arginine solution. Since this involves the i.p. injection of relatively large amounts of fluid, first we wanted to characterize the effects of various L-arginine-HCl concentrations (5 %, 8 %, 10 % and 30 %) on the development of AP in BALB/c mice. According to preliminary experiments, the administration of 30 % L-arginine-HCl solution greatly increased the need of euthanasia (over 90 % in all mouse strains), so we did not proceed with this concentration any further. Interestingly, whereas treatment of mice with 2×4 g/kg 5 % L-arginine-HCl solution did not cause any mortality or pancreatic damage (therefore, its effectivity rate was 0) in the BALB/c strain, the effectivity rate of the 8 and 10 % L-arginine-treated groups was over 90 %.

AP developed in the 8 and 10 % L-arginine-treated groups, whereas no AP was seen in the 0 and 5 % treated groups. Notably, the extent of acinar cell damage was relatively mild in pancreatitic animals. There were no significant differences in the measured parameters of the 8 and 10 % treated groups, so to decrease the volume load, we used a 10 % L-arginine-HCl solution for further studies.

Next, we tested the effects of L-arginine-HCl concentration on the C57BL/6 mouse strain. Animals were treated with PS or with 2×4 g/kg, 5-10 % L-arginine-HCl solution. Although the effectivity rate of AP induction was 93 % in the 5 % L-arginine-treated group, it was only 33 % in the 10 % L-arginine-treated group. The low effectivity rate in the latter group was caused by the high euthanasia rate. Mice that were euthanized 12 h after the injections of L-arginine (not related to AP induction), or L-arginine-treated mice that had no pancreatic inflammation on histology at the time of sacrifice were excluded from the data analysis.

Both laboratory and histological parameters confirmed the presence of AP in the groups treated with 5 and 10 % L-arginine vs the PS-treated control group. AP was more severe in the 10 % vs 5 % L-arginine-treated group based on laboratory and histological parameters.

Next, we administered 2×4 g/kg 5-10 % L-arginine-HCl intraperitoneally (i.p.) in FVB/n mice. Similarly to that observed in C57BL/6 mice, in the FVB/n mouse strain the effectivity rate in the 5 % L-arginine-treated groups was 92 %; however, it was only 25 % in the 10 % L-arginine-treated group. Laboratory and histological parameters were significantly elevated in the 5 and 10 % L-arginine-treated groups, compared to the control group. However, there were no significant differences between the 5 and 10 % L-arginine-treated groups. Administration of 5 and 10 % L-arginine caused similar degree of pancreatic inflammation.

Dose-response of L-arginine administration in C57BL/6 and FVB/n mice

BALB/c mice proved to be rather resistant against mortality and AP injury caused by i.p. injection of 2×4 g/kg L-arginine-HCl, whereas the other two mouse strains seemed to be much more susceptible.

We figured that by using other L-arginine doses, we can reduce adverse effects and increase pancreatic damage in C57BL/6 and FVB/n mice. 2×4, 3×3, or 4×2.5 g/kg L-arginine-HCl (10%) in C57BL/6 mice were administered to determine the dose-response of L-arginine administration. The 2×4 g/kg L-arginine-HCl dose resulted in a low effectivity rate, because of the numerous euthanized mice. In contrast, the effectivity of AP induction in response to administration of 3×3 g/kg or 4×2.5 g/kg L-arginine-HCl was significantly higher vs the 2×4 g/kg L-arginine-HCl treated group. The severity of AP was not significantly different in the L-arginine-treated groups according to histological and laboratory parameters. Of note, the i.p. injection of 5×2 g/kg L-arginine-HCl at hourly intervals did not cause any signs of AP. We found similar dose-response (2×4, 3×3, or 4×2.5 g/kg L-arginine-HCl) results in FVB/n mice. There were no significant differences between the effects of different L-arginine doses on the severity of AP.

The severity of L-arginine-induced acute pancreatitis is similar in male and female mice

As some AP models show gender dependency (e.g. female mice are more susceptible to CDE diet-induced AP), we wanted to test the effects of L-arginine administration in male and female mice. C57BL/6 mice were subjected to hourly i.p. injections of 3×3 g/kg 10 % L-arginine-HCl. The effectivity rate of AP induction was 87.5 % in male and 100 % in female mice, which was not significantly different.

The severity of fatty acid and ethanol-induced AP in mice

Previously, Maléth *et al. (2015)* have shown that the mixture of 0.8 g/kg ethanol and 300 mg/kg PA time-dependently decreased the expression of CFTR in pancreatic ductal epithelial membrane in guinea pigs, however, the expression was increased in the cytoplasm. Based on *in vitro* data, we concluded that the ethanol and fatty acid cocktail may cause the damage and internalization of CFTR. Former studies suggested that decreased CFTR expression causes reduced pancreatic ductal fluid secretion and a more severe pancreatitis. In our *in vivo* experiments, mice were treated with 1.75 g/kg ethanol and 750 mg/kg PA, which caused acute necrotizing pancreatitis. We tested the fatty acid and ethanol-induced AP model in WT and also in CFTR KO mice. We found that the measured laboratory (pancreatic water content, serum amylase activity) and histological parameters (oedema, leukocyte infiltration score, necrosis %) were significantly elevated in CFTR KO mice compared to WT.

Discussion

L-arginine-induced AP

An important finding of our experiments is it that different mouse strains have varying sensitivities (resulting in death, AP, or no pancreatic damage) to administration of L-arginine-HCl (pH=7.4). In FVB/n and C57BL/6 mice, which are commonly used strains for generating transgenic animals, the originally published i.p. 2×4 g/kg L-arginine-HCl dose caused great mortality and the use of other doses (like 3×3 g/kg or 4×2.5 g/kg) may be better for the induction of AP. Furthermore, the concentration of the administered L-arginine-HCl solution makes a huge difference in whether the animals survive the treatment. High L-arginine-HCl concentration (30 %), which are well tolerated by rats actually kill mice, thus lower (5-10 %) concentrations should be used even if this means considerably more fluid volume. Interestingly, no significant differences were detected in AP severity of male and female mice.

Considering the survival of animals, the sensitivity of FVB/n and C57BL/6 mice to L-arginine administration was much higher than that of BALB/c mice. In fact, it seems that the most L-arginine resistant mouse strain is the BALB/c. In this strain, we found no mortality in response to i.p. injection of 2×4 g/kg L-arginine-HCl. In accord with our observations, Dawra

et al. (2007) (who have also used BALB/c mice) reported no mortality in their original study. In contrast, we had to use euthanasia in a great proportion of FVB/n and C57BL/6 mice after the i.p. administration of 2×4 g/kg L-arginine-HCl. The cause of death was unrelated to AP and may be due to severe metabolic acidosis detected in rats by Bohus *et al*. or central nervous system effects. The reasons for the above mentioned discrepancies are not clear, but the dose response of rats to i.p. administration of L-arginine and L-lysine can be variable even in one animal strain, although no such effects have been reported in mice. Sprague-Dawley rats treated with a high dose (4 g/kg) of i.p. L-arginine caused weak, normal and strong response. Weak responders had no marked alterations vs the saline-treated control group, whereas strong responders died. In our hands, the administration of 3.5 g/kg L-arginine causes marked pancreatic inflammation and cell damage, but no mortality; however, we also observed a small percentage of mortality with the 4 g/kg L-arginine-HCl dose in male Wistar rats (unpublished data). Similarly to the data of Bohus *et al*. (2008), the i.p. injection of large L-lysine doses caused nil to severe pancreatic damage or death of rats. Based on the above mentioned data, we speculate that there may be differences in basic amino acid sensitivities even within the same mouse strain.

Most commonly, an i.p. dose of 2×4 g/kg L-arginine-HCl is used for the induction of AP in mice, which was initially used in our study as well. However, the utilization of higher, and some rather odd L-arginine doses have also been reported. Previously published L-arginine doses ranging from 1×0.000004 g/kg to 2×2.25 g/kg administered in mice do not cause any signs of AP in our hands. Similarly, Dawra *et al*. (2007) could not detect any significant changes in serum amylase activity, and pancreatic MPO activity and histopathology in response to i.p. 2×2 g/kg or 2×3 g/kg L-arginine-HCl. However, we have clearly shown that the administration of 3×3 g/kg or 4×2.5 g/kg L-arginine-HCl induced severe AP in the majority of FVB/n and C57BL/6 mice, but caused a marked reduction of mortality vs the 2×4 g/kg dose. When we administered the same L-arginine-HCl doses in the three mouse strains, AP severity was nearly the same in FVB/n and C57BL/6 mice, but it was markedly less in BALB/c mice. The strain-dependent susceptibility to cerulein- and CDE diet-induced AP was reported by Wang *et al*. (2010). The severity of experimental pancreatitis in their study was moderate in BALB/c and mild in C57BL/6J mice. This difference in susceptibility was attributed to a positive relationship with proteinase, serine, 1 expression and a negative relationship with serine protease inhibitor, Kazal type 3 expression. In L-arginine-induced AP, this may not be the case since disease severity was significantly higher in C57BL/6 vs BALB/c mice.

The effects of the estrous cycle on AP development have always been a concern of researchers, thus to exclude such potential effects, usually male animals are used. In fact, it has long been known that in case of the CDE diet model, the effects are gender specific, female mice are much more sensitive to treatment. Therefore, we also tested the potential gender-specific effects of L-arginine treatment, but we found no significant differences in AP severity of male vs female C57BL/6 mice.

The exact pathomechanism of basic acid-induced AP is not well understood, maybe the metabolites of L-arginine have an important role in pancreatitis induction. In fact, there are two key enzymes involved in the metabolism of this semi-essential amino acid: nitric oxide synthase and arginase.

Taken together, it is evident that setting up the L-arginine-induced AP model in mice is quite challenging. Overall, it seems that the borderline between the effective (AP inducing) and lethal doses of L-arginine is much thinner in mice vs rats. There are several important factors that need to be considered such as the concentration and dose of the injected L-arginine-HCl solution and also the strain of mice. The proper dosing of L-arginine to induce AP should be tested by each laboratory and in each mouse strain. The reasons for the differences in L-arginine sensitivity of mice remain to be investigated. L-arginine-induced AP could be a cheap, reproducible, non-invasive, easy to use in rats and also in mice. The use of this model in mice is important because of the widespread transgenic animals.

The effects of CFTR inhibition on the severity of fatty-acid and ethanol-induced acute pancreatitis

In 1965, Henry Sarles *et al*. suggested that patients suffering from alcohol-induced AP have similar alterations in pancreatic juice and the composition of sweat like some cystic fibrosis patients. These were the first evidences that CFTR channel and pancreatitis have some connections.

It was also demonstrated, that CFTR mutations not only cause cystic fibrosis, but they are risk factors for pancreatitis. Association between *CFTR* gene mutations and the risk for the development of acute recurrent or chronic pancreatitis provided strong evidence that mutations in CFTR and insufficiency of electrolyte and fluid secretion by pancreatic ductal cells lead to increased risk for pancreatitis. Heterozygous carriers of *CFTR* mutations have an increased risk of chronic pancreatitis. Furthermore, demonstrated that the risk of developing pancreatitis was

much higher in CF patients, who had milder *CFTR* mutations and were pancreatic sufficient compared to those who had severe mutations and were pancreatic insufficient. 15-20 % of patients suffering from cystic fibrosis may present with recurrent AP. Heterozygous carriers of del508F *CFTR* mutation have an elevated risk and odds ratio for pancreatitis ~7 % and ~2.5, respectively. Heterozygous carriers of mild CFTR mutations, like R117H also have an elevated risk and odds ratio for pancreatitis, compered to healthy people (~2.5 %, ~4).

Following the development of genetically modified animals, the research of CFTR became easier. DiMagno *et al*. showed in 2005, that CFTR KO mice had a reduced stimulated biliopancreatic secretory rate compared to their WT littermates. They induced AP with repetitive administration of cerulein, investigated laboratory and histological parameters and found that pancreatic damage was more severe in CFTR KO mice compared to WT mice.

Pallagi *et al*. (2014) investigated the role of Na^+/H^+ exchanger regulatory factor-1 (NHERF-1) in experimental AP. NHERF-1 is a scaffolding protein involved in the apical targeting and trafficking of several membrane proteins and connects them to the cytoskeleton. Without the proper function of NHERF-1, many transporter and channel functions, like that of CFTR are diminished. It was shown that in NHERF-1 KO mice, pancreatic ducts had reduced CFTR expression compared to their WT littermates. It was found that HCO_3^- and fluid secretion *in vitro* on isolated pancreatic ductal epithelial cells and also *in vivo* were reduced in NHERF-1 KO mice. Animals were investigated during inflammatory conditions and they found that in cerulein and also in ductal bile acid infusion model AP was more severe in NHERF-1 KO mice compared to their WT littermates.

Maléth *et al*. (2015) demonstrated that ethanol and fatty acid cocktail in high concentration decreased the CFTR expression *in vitro* on CAPAN-1 cell line and also *in vivo* in guinea pigs. They also confirmed with magnetic resonance cholangiopancreatography examination that the pancreatic secretory rate is decreased after ethanol and PA treatment. Based on these data, we found that ethanol and palmitic acid-induced AP was more severe in CFTR KO mice compared to their WT littermates.

Our data that ethanol and fatty acid-induced AP is more severe in CFTR KO mice was well correlated with the former literature data and confirm the crucial role of pancreatic ductal secretion in the pathogenesis of alcohol-induced pancreatitis. It is evident that CFTR has an important role in pancreatic dcutal secretion and the pathogenesis of AP and it may be a therapeutic target in the future.

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"Per aspera ad astra" (Latin phrase)