

Effects of alcohol on pancreatic ductal function

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1. Importance of the pancreatic ductal HCO₃⁻ secretion

The exocrine pancreas secretes ~ 1.5 L of alkaline, isotonic fluid, which washes the digestive enzymes from the lumen of the pancreatic ducts and neutralizes the acidic gastric content entering the duodenum (4, 24). This alkaline pancreatic secretion plays an important role in the physiology and pathophysiology of the gland protecting the pancreatic tissue from damage. Findings from the last two decades supported this hypothesis and highlighted that the pancreatic acinar cells will suffer severe damage, if the pancreatic ductal secretion is impaired. Freedman et al. observed that in *cftr* knockout mice the pancreatic ductal secretion is impaired resulting in a more acidic (pH 6.6±0.04) pancreatic juice compared to wild type animals (pH 8.12±0.06) (13). In addition, the lack of cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel activity caused a defect in the apical membrane transport of the acinar cells. The findings of Reber et al. showed that in cat pancreas, the basal parenchymal pH was ~7.35, which decreased to ~7.25 after the induction of chronic pancreatitis (35). Moreover, ethanol administration decreased the extracellular pH of the pancreatic tissue to

~7.1 and reduced pancreatic blood flow to 40%. In a rat model, the development of acute pancreatitis (AP) was affected by the pH of the contrast solution during endoscopic retrograde cholangiopancreatography (28). Contrast solution at pH 6.0-6.9 injected into the main pancreatic ducts induced pancreatic oedema, increased serum amylase activity, neutrophil infiltration, and histological damage. The pancreatic injury correlated with the lower pH. On the other hand, pH 7.3 solution caused only mild pancreatic injury. Bhoomagoud et al. showed that the decrease of the extracellular pH from 7.6 to 6.8 augmented secretagogue-induced zymogen activation and acinar cell injury *in vitro* and enhanced cerulein-induced trypsinogen activation and pancreatic oedema *in vivo* (5). Our group further proved the importance of the pancreatic ductal secretion, since we demonstrated that the autoactivation of trypsinogen is a pH dependent process, with accelerated autoactivation on acidic pH meaning that HCO₃⁻ secretion protects the pancreas from untimely trypsinogen autoactivation (31). Evidence suggests that the decreased pancreatic ductal bicarbonate secretion can affect the severity of AP (see below).

2. Mechanism of the bicarbonate secretion in pancreatic ductal cells

The major site of the fluid and HCO_3^- secretion are the pancreatic ductal epithelial cells (PDEC) of the small intercalated and intralobular ducts (6). The maximal HCO_3^- concentration in the ductal lumen can vary among species; importantly human PDEC can produce 140 mM maximal intraluminal HCO_3^- concentration as can guinea pigs (4).

The complex process of pancreatic ductal HCO_3^- secretion can be divided to two steps, the accumulation of HCO_3^- across the basolateral membrane followed by the secretion via the apical membrane into the lumen. The basolateral accumulation is mediated by the $\text{Na}^+/\text{HCO}_3^-$ cotransporter (NBCe1-B), which operates with 1 Na^+ and 2 HCO_3^- stoichiometry (17). The passive diffusion of CO_2 through the basolateral membrane may also contribute to the HCO_3^- accumulation, which is followed by the carbonic anhydrase mediated conversion of CO_2 to HCO_3^- (12). On the luminal membrane of the PDEC the molecule central to HCO_3^- secretion are the electrogenic $\text{Cl}^-/\text{HCO}_3^-$ exchangers (SLC26A6 and possibly A3, which operates with a 1 Cl^- : 2 HCO_3^- stoichiometry) (39). Another important protein is the CFTR Cl^- channel, which plays an important role in the ductal HCO_3^- secretion in humans and animals, which produce a high intraluminal HCO_3^- concentration (45). This electrogenic apical $\text{Cl}^-/\text{HCO}_3^-$ exchange allows PDEC to transport HCO_3^- into the ductal lumen and establish 140 mM intraluminal HCO_3^- concentration during stimulated secretion (4, 24). The details and the molecular background of the pancreatic ductal HCO_3^- secretion have been reviewed recently elsewhere (1, 24, 27).

3. Effects of ethanol and ethanol metabolites on the pancreatic ductal bicarbonate secretion

One of the most common causes of AP is heavy alcohol abuse. The inhibitory effect of alcohol on the pancreatic secretion was first suggested decades ago (16). In experimental studies Yamamoto et al. found that 0.3-30 mM ethanol augmented, whereas 100 mM ethanol inhibited secretin-stimulated pancreatic ductal fluid secretion in the guinea pig (44). In this study the authors focused on the effects of ethanol; however, numerous studies have highlighted the harmful effects of different ethanol metabolites in different organs. *In vivo* ethanol metabolism is carried out by two independent pathways (23, 33). The oxidative pathway happens dominantly in the liver and generates acetaldehyde, whereas, the non-oxidative pathway combines ethanol and fatty acids (FA) and produces fatty acid ethyl esters (FAEE) in the pancreas, brain and heart, tissues typically damaged by excessive ethanol consumption (23). Compared with the liver, FAEE synthase activity in the pancreas is greater creating the possibility for the local accumulation of non-oxidative ethanol metabolites (15). FAEE can also be hydrolyzed leading to the intracellular accumulation of FA, which can strongly bind to mitochondrial membrane proteins and thus uncouple oxidative phosphorylation (22). Clinical studies (43) and experimental animal models suggest that ethanol administration *in vivo* does not induce pancreatitis by itself but sensitizes the pancreas to other triggers (32). Ethanol was shown to destabilize lysosomes and zymogen granules (42), sensitize pancreatic mitochondria to activate mitochondrial permeability transition pore (MPTP) leading to mitochondrial failure (38), modulate the immune response via sensitizing NF- κ B activation in pancreatic acinar cells (37) and cause oxidative ER stress, which activates an unfolded protein response and increases XBP1 levels and activity (25). Criddle et al. found that FAEE and FA, however, not ethanol cause pancreatic acinar cell damage via sustained

intracellular Ca^{2+} elevation, mitochondrial dysfunction, ATP depletion and intraacinar trypsinogen activation leading to cell necrosis (7, 8, 14, 34). Ethanol metabolites were also shown to perturb exocytosis processes in cultured rat pancreatic acini causing apical blockade and basolateral exocytosis (11). Moreover, Werner et al. showed that FAEE infusion induced significant increases in pancreatic edema, pancreatic trypsinogen activation, and vacuolization of acinar cells (41). Recently the role of stellate cell activation has also been highlighted in the ethanol induced pancreatic injury (3); however, there is no direct evidence concerning the involvement of ductal epithelial cells in the pathogenesis of alcohol-induced pancreatitis.

Importantly, Sarles et al. described that the initial lesion in course of pancreatic damage during alcohol-induced chronic calcifying pancreatitis is the formation of mucoprotein plugs in the small pancreatic ducts (36). Besides this, the sweat chloride and sodium concentration of these patients were also significantly elevated compared to the control group (36). These changes are very similar to the alterations of the exocrine pancreas in cystic fibrosis, the most common genetic mutation in the Caucasian population, which was shown to associate with exocrine pancreatic insufficiency (21) and increased risk of pancreatitis (29). Although the observations of Sarles are more than 50 years old, the connection of ethanol induced pancreatic damage and ductal secretory dysfunction has not been investigated in details yet.

Recently we demonstrated using several overlapping *in vivo* and *in vitro* experimental models that ethanol and FA dose-dependently reduced CFTR expression and activity in PDEC, and inhibited secretion of fluid and HCO_3^- in the pancreas (18, 26). We observed that the sweat Cl^- concentration (Cl_{sw}^-) was significantly elevated after heavy alcohol intake in human subjects; however, the Cl_{sw}^- normalized when the patients were sober (26). In human tissue samples from patients suffering from alcohol-induced acute or

chronic pancreatitis we detected a significant decrease of the CFTR expression at the apical membrane of the pancreatic ducts. Interestingly in experimental models we found that low concentration (10 mM) of ethanol stimulated both the apical $\text{Cl}^-/\text{HCO}_3^-$ exchange and the CFTR channel activity. However, at high concentration (100 mM) a strong inhibitory effect was detected on the HCO_3^- secretion, CFTR activity and pancreatic fluid secretion *in vivo* and *in vitro*. This dual effect of ethanol is very similar to the dose-dependent effects of non-conjugated bile acids on the pancreatic ductal functions (40). Similarly to 100 mM ethanol, FA augmented the pancreatic fluid and HCO_3^- secretion. The oxidative ethanol metabolite acetaldehyde and FAEE have no such effects. The inhibition of CFTR by ethanol and FA was associated with a sustained increase in concentrations of intracellular Ca^{2+} and decreased 3',5'-cyclic adenosine monophosphate (cAMP) levels, mitochondrial membrane depolarization, and a consequent drop of intracellular ATP. Intracellular ATP supplementation via a patch pipette almost completely prevented inhibition of CFTR activity by ethanol and FA (18). We also showed that the decrease in CFTR expression and plasma membrane density in response to ethanol, palmitoleic acid, or palmitoleic acid ethyl ester administration was caused by the combination of accelerated plasma membrane turnover at the apical membrane and by damaged protein folding in the endoplasmic reticulum (26).

4. Alcohol-induced CFTR dysfunction in the pathogenesis of pancreatic damage

As demonstrated above ethanol and ethanol metabolites have a strong inhibitory effect on the pancreatic HCO_3^- and fluid secretion via the reduced function and expression of CFTR (Figure 1). In addition to these experimental observations, other data suggest that CFTR function can affect the pathogenesis and severity of acute pancreatitis.

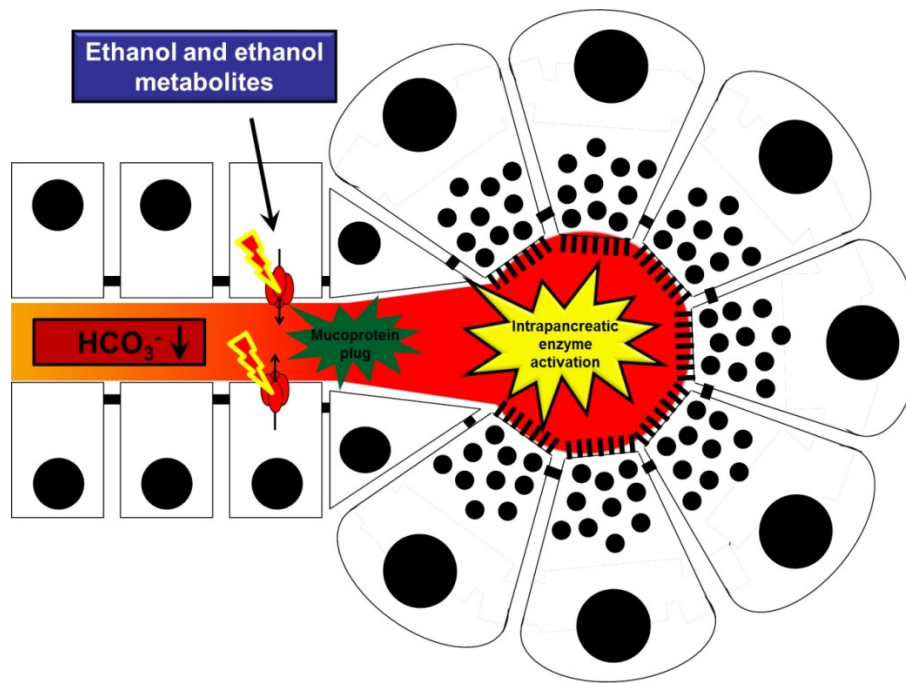


Figure 1. The effects of ethanol and ethanol metabolites on pancreatic ductal function. Under physiological conditions, CFTR Cl^- channel (red) is expressed on the luminal membrane of small inter/intralobular pancreatic ducts and contributes significantly to the pancreatic HCO_3^- secretion, which maintains the alkaline intraluminal pH. During acute or chronic alcohol-induced pancreatitis the function and expression of CFTR is markedly reduced by ethanol and ethanol metabolites, which leads to impaired HCO_3^- and fluid secretion and consequently decreased intraluminal pH. Under these conditions the wash out of the luminal content is insufficient promoting the formation of intraluminal protein plugs. The intraductal obstruction will lead to intrapancreatic enzyme activation in acute pancreatitis and to pancreatic atrophy and exocrine pancreatic insufficiency in chronic pancreatitis.

DiMagno et al. showed that deletion of CFTR results in continuous overexpression of proinflammatory cytokine genes, moreover these mice develop more severe acute pancreatitis upon cerulein hyperstimulation compared to wild type (9). They observed elevated pancreatic edema, neutrophil infiltration and mRNA expression of multiple inflammatory mediators; however, acinar cell injury was not different. On the other hand acinar cell apoptosis was decreased in CFTR knockout mice, which also had mild exocrine pancreatic insufficiency (as pointed out by impaired *in vivo* pancreatic secretion in response to cholecystokinin and reduced pancreatic digestive enzyme protein and mRNA levels). These results were reproduced in ΔF508 mutant mice (10). These observations are important, although the authors focused on the alterations of acinar cells, whereas CFTR is expressed on the apical membrane of pancreatic

ductal cells. The lack of pancreatic CFTR expression impairs the ductal fluid and bicarbonate secretion and any alterations of the acinar cells might be presumably indirect. Recently, our group demonstrated that CFTR knockout mice displayed more severe acute pancreatitis induced by i.p. injection of ethanol and palmitic acid (26). All laboratory and histological parameters were significantly elevated in CFTR knockout mice compared to wild type controls, including the extension of necrosis. These data have potential clinical relevance as well, since we detected markedly decreased CFTR protein and mRNA expression in small pancreatic ducts using pancreatic tissue samples from patients diagnosed with alcohol-induced acute pancreatitis (26). Another study by Pallagi et al. confirmed the potential role of CFTR and pancreatic ductal secretion in the pathogenesis of acute pancreatitis (30). In this study, Na^+/H^+

exchanger regulatory factor-1 (NHERF-1, a cytosolic scaffolding protein involved in the apical targeting and retention of membrane proteins) knockout mice were used, which had lower CFTR expression in the apical membrane of pancreatic ducts and lower pancreatic bicarbonate and fluid secretion. Serulein hyperstimulation and sodium taurocholate infusion into the pancreas induced more severe pancreatitis further confirming the importance of CFTR-mediated pancreatic secretion.

On the other hand, alcohol-induced CFTR dysfunction and therefore impaired HCO_3^- secretion seems to be involved not just in the pathogenesis of AP, but in chronic pancreatitis (CP) as well. In CP the destruction of the pancreas can be observed due to chronic inflammation, exocrine pancreatic insufficiency, decreased pancreatic fluid and bicarbonate secretion, fibrosis and calcification of the tissue. As an underlying mechanism for the decreased secretion, CFTR dysfunction due to mislocalised protein expression in pancreatic ductal cells has been observed in different forms of CP. Using

human pancreatic tissue samples Ko et al. described that CFTR is mislocalised in alcoholic, obstructive and idiopathic chronic pancreatitis as well similarly to our results (20). The decreased expression of CFTR, observed in different forms of chronic pancreatitis, could explain the impaired function of the PDEC (20). The impaired fluid and HCO_3^- secretion lead to decreased intraluminal pH, decreased wash out of the digestive enzymes and more viscous, protein-rich ductal fluid (**Figure 1**) (19). These changes promote the formation of intraluminal protein gel, or plugs that are one of the earliest histological features of chronic pancreatitis (36). The intraductal obstruction can lead to pancreatic atrophy, ductal mucinous hyperplasia (2), Goblet-cell metaplasia and the protein plugs might also underlie pancreatic stone formation (19).

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