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Alcohol and Smoking in Chronic Pancreatitis

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Introduction

Chronic pancreatitis (CP) develops resulting from interactions of multiple environmental, metabolic, and genetic risk factors [1]. Alcohol is the leading cause for CP in many Western countries, and the association between alcohol misuse and CP has been recognized for a long time. As early as 1878, Friedreich [2] described "drunkard's pancreas": chronic interstitial inflammation in the pancreas which might result from alcohol misuse. In 1946, Comfort et al. [3] described the clinical presentation of chronic relapsing pancreatitis in subjects with alcohol misuse. Thereafter, it has been established that alcohol misuse is an important risk factor for CP. Of note, unlike the alcohol-induced liver injury, only 1–5 % of heavy drinkers develop pancreatitis [4], indicating that alcohol-related pancreatitis is not caused by alcohol misuse alone. Some individuals may develop alcohol-related pancreatitis with alcohol intake as low as 20 g/day, whereas most individuals do not develop pancreatitis no matter how much they drink or how long. In animals, ethanol feeding alone does not cause a pronounced pancreatic injury. Therefore, additional genetic and/or environmental predisposing factors are required for the development of clinical CP.

Conversely, the independent effects and risks associated with smoking have not been well recognized until recently. Because smoking is strongly associated with drinking alcohol [5,6], the independent effect of smoking can be difficult to assess. Smoking is now recognized to confer a strong, independent and dose-dependent risk of CP [5–7]. Importantly, smoking and alcohol interact and worsen acinar cell injury and pancreatitis synergistically [5,6]. In this chapter, we review the clinical observations and pathophysiology of alcohol-related and smoking-related CP.

Alcohol and Chronic Pancreatitis

Clinical Observations

Historically, alcohol misuse is the leading cause of CP and accounts for approximately 60–90% of CP cases in industrialized nations worldwide [6]. However, in recent years, the proportion of alcohol-related CP (ACP) cases might be smaller than expected. The North American Pancreatitis Study 2 (NAPS2) showed that the frequency of ACP at tertiary referral centers in the United States was 44.5% [6]. A report from Italy showed a shift in the etiologic profile of CP [8]. Alcohol misuse was the leading cause of CP (74%) between 1971 and 1995, but the proportion was decreased to 43% in patients evaluated between 2000 and 2006. These findings suggest that the contribution of alcohol misuse to the pathogenesis of CP might have been overestimated [6]. Referral bias might exist in tertiary referral centers and accurate assessment of alcohol exposure to determine the association with CP is challenging because self-reports about alcohol consumption are usually unreliable. Conversely, in Asia, ACP accounted for 72% of all CP cases in Japan in 2016 [9]. In India and China, the proportion of ACP was lower and idiopathic pancreatitis was the most common type, accounting for approximately 70% of the CP cases [10]. Importantly, alcohol consumption has been stable or decreasing in many North American and European countries as well as in Japan, whereas it has been increasing in India and China [10]. It would be of interest to see whether the trends in alcohol consumption affect the burden of ACP in India and China in the future.

There have been many studies that aimed to clarify the dose–response relationship between alcohol consumption and CP. The association between alcohol

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consumption and pancreatitis was evaluated in 540 cases and 695 controls in the NAPS2 [5]. Logistic regression analyses revealed a significant association between alcohol and CP only in very heavy drinkers who consumed ≥5 alcoholic drinks per day (odds ratio [OR] = 3.1). In another case-control study [11], among patients with onset of CP after the age of 35, alcohol intake, even less than 50 g/day, induced earlier disease characterized by more frequent severe pain, calcification, and complications such as pseudocysts. In a Japanese case-control study [12], compared with nondrinkers, the OR (95% confidence interval [CI]) for alcohol consumption of 20≤~<40 g/day, 40≤~<60 g/day, 60≤~<80 g/day, 80≤~<100 g/day, and ≥100 g/day were 2.6 (1.2–5.5), 3.2 (1.5–7.1), 9.2 (4.1–20.3), 13.0 (5.3–31.6), and 19.6 (8.2–46.8), respectively. A systematic review and meta-analysis of four studies (three case-control and one cohort studies) showed that the risk of CP increased monotonically according to the average alcohol consumption with no identifiable threshold in men [13]. The relative risks (RR) (95% CI) were 1.58 (1.32–1.90) at 25 g/day; 2.51 (1.74–3.61) at 50 g/day; 3.97 (2.30–6.85) at 75 g/day; and 6.29 (3.04–13.02) at 100 g/day.

It is well known that ACP is predominantly a disease of men [5,9]. However, alcohol misuse is an important health problem in women, too. It has been shown that susceptible women might develop ACP with shorter duration and lower cumulative amounts of alcohol consumption than men [14].

Ethanol Metabolism in the Pancreas

Ethanol can be metabolized in the pancreas, mainly in pancreatic acinar cells. Two pathways of ethanol metabolism have been described in pancreatic acinar cells: oxidative and nonoxidative pathways [15]. Ethanol oxidation involves the conversion of ethanol to acetaldehyde and acetate, a reaction catalyzed by ADH and cytochrome P450 2E1. The nonoxidative pathway of ethanol metabolism involves the esterification of ethanol with fatty acids to form fatty acid ethyl esters (FAEE) such as palmitic acid ethyl ester. This reaction is catalyzed by FAEE synthases. FAEE synthase activity in the pancreas is much greater than that in the liver, whereas pancreatic ADH and cytochrome P450 2E1 activities are low [15]. Therefore, the dominant nonoxidative metabolism is a characteristic feature of ethanol metabolism in the pancreas.

Pathophysiology

Several pathophysiological mechanisms linking alcohol consumption and pancreatitis have been suggested. At the cellular level, ethanol and its metabolites affect the cell functions and homeostasis of pancreatic cells including acinar cells, ductal cells, and stellate cells (Table 48.1) [16,17 and references therein]. Importantly, animal studies have suggested that ethanol alone does not induce pancreatitis unless additional pathogenic insults are present. One explanation would be that the pancreas can compensate harmful effects of alcohol through an adaptive stress response. Pancreatitis develops only when the compensatory mechanisms are disrupted/exhausted or if increased vulnerability due to other genetic and environmental cofactors are present. Findings in animal studies support the concept that alcohol misuse alone does not cause CP and additional cofactors are required for the development of CP in susceptible humans.

Table 48.1 Effects of ethanol on pancreatic cells [16,17].

1. induces mitochondrial damage
2. elevates intracellular calcium levels
3. disrupts expression and function of the CFTR
4. decreases bicarbonate secretion
5. increases pancreatic digestive enzyme content
6. redirects exocytosis to the basolateral surface
7. enhances pancreatitis responses elicited by hyperstimulation
8. induces endoplasmic reticulum stress
9. promotes oxidative stress
10. increases the fragility of lysosomes and zymogen granules
11. regulates transcription factors NF-κB and AP-1
12. activates stellate cells to promote fibrosis

CFTR: cystic fibrosis transmembrane conductance regulator; NF-κB: nuclear factor-κB; AP-1: activator protein-1.

Pancreatic Acinar Cells

Ethanol induces a sustained elevation of the intracellular calcium levels, which is central in promoting pathological events of pancreatitis. FAEE cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of adenosine triphosphate production [18]. Calcium overload reduces adenosine triphosphate production and subsequently causes dysfunction of endoplasmic reticulum (ER). Ethanol increases the fragility of zymogen granules and lysosomes, which sequester lysosomal enzymes such as cathepsin B within the cells. Oxidative alcohol metabolism induces mitochondrial dysfunction, which might play a role in ethanol-induced necrosis of the pancreatic acinar cells [19]. Mitochondrial dysfunction occurs due to membrane permeabilization mediated by persistent opening of the mitochondrial permeability transition pore, leading to loss of mitochondrial membrane potential and mitochondrial fragmentation.

These changes might make the pancreas susceptible to necrotizing pancreatitis.

Chronic ethanol feeding in mice perturbs protein folding and induces ER stress, which in turn induces an unfolded protein response (UPR) involving the upregulation of spliced X box-binding protein 1 (sXBP1) in pancreatic acinar cells [20]. Ethanol administration to mice heterozygous for XBp1 ($XBP1^{+/-}$) resulted in dilated ER, loss of zymogen granules, accumulation of autophagic vacuoles, and acinar cells death, suggesting that these responses serve as a protective adaptive mechanism that prevents ethanol-induced damage.

Autophagy is impaired in pancreatitis and lysosome dysfunction might be involved. Autophagy comprises several intracellular pathways of lysosome-mediated degradation and recycling of organelles, long-lived proteins and lipids [21]. Fortunato et al. [22] reported that the combination of ethanol exposure and endotoxemia resulted in the depletion of several lysosomal proteins including lysosomal-associated membrane protein-2 (LAMP-2), a protein required for the proper fusion of autophagosomes with lysosomes. Cathepsin B-induced LAMP degradation and genetic LAMP-2 deletion caused pancreatitis through impaired autophagy in mice [23]. Human patients with alcoholic pancreatitis also exhibited local LAMP-2 depletion, indicating the crucial roles of LAMP-2 and autophagy in acinar cell death in humans.

Although numerous *in vitro* and *ex vivo* studies have shown the actions of ethanol and its metabolites on pancreatic cells, *in vivo* studies have shown that feeding ethanol to rats and mice even for a long time, with either liquid diet or continuous intragastric infusion, did not cause a prominent injury to the pancreas. Chronic ethanol feeding by the Lieber-DeCarli pair-feeding model [24] induces a number of metabolic changes in the acinar cells including an increase in the content of digestive enzymes and lysosomal enzyme and fragility of the zymogen granules and lysosomes. However, chronic pathological changes resembling CP will not develop. Conversely, chronic ethanol exposure sensitizes the pancreas to other insults. Pancreatitis developed in rats that had received an ethanol-containing diet in response to low doses of cholecystokinin octapeptide or its analogue caerulein, which do not cause pancreatitis by themselves [25]. The sensitization was accompanied by increased activation of nuclear factor-κB (NF-κB) and the upregulation of proinflammatory cytokines and chemokines in the pancreas [26]. Ethanol might regulate NF-κB and activator protein (AP)-1: the key transcription factors regulating the gene expression of inflammatory responses and cell survival. FAEE activate NF-κB and AP-1, whereas ethanol and acetaldehyde inhibit NF-κB activation [26]. Thus, ethanol may regulate the activation of NF-κB and AP-1 positively or negatively, depending on the predominance of which metabolic pathway's effects. These effects may play a role in the ethanol-induced toxicity in the pancreas. Of note, ethanol and its metabolites altered the cholecystokinin 8-induced activation of these transcription factors, which might be a mechanism by which ethanol sensitizes pancreatic acinar cells to pancreatitis.

Gukovsky et al. [27] reported that ethanol dramatically aggravated the pathological effects of the combination of cyclosporine A and caerulein. In ethanol-fed, but not control diet-fed, animals, the combined treatment of cyclosporine A and caerulein resulted in severe pancreatic injury that displayed three key responses of human ACP: loss of parenchyma, sustained inflammation, and fibrosis. Conversely, for the repair of the exocrine pancreas, acinar cells could act as progenitor cells; mature acinar cells undergo dedifferentiation and redifferentiation back to the differentiated phenotype [28]. Clemens et al. [29] reported that chronic ethanol administration delayed the structural and functional regeneration of the pancreas in mice. The delayed regeneration was associated with the decreased expression of pancreatic developmental factors including PDX-1. These findings suggest that ethanol might impair the recovery from acute pancreatic injury, thus facilitating the progression from acute pancreatic injury to CP.

Pancreatic Ductal Cells

Alcohol increases viscosity and precipitation of pancreatic juice and formation of protein plugs inside pancreatic ducts. Protein plugs lead to formation of calculi which damage ductal epithelium and cause obstruction of pancreatic ducts [30]. Several studies have highlighted the role of the pancreatic duct in the pathogenesis of alcohol-induced pancreatitis. Ethanol reduced the expression of cystic fibrosis transmembrane conductance regulator (CFTR), disrupted the folding of CFTR at the ER, and inhibited CFTR function in pancreatic ductal cells, all contributing to the increased viscosity of the pancreatic juice and small duct obstruction [31]. *CFTR* knockout mice given ethanol or fatty acids developed more severe pancreatitis than mice not given ethanol or fatty acids.

Pancreatic Stellate Cells

PSC play a pivotal role in pancreatic fibrosis in CP. Ethanol and its metabolites induced the activation, extracellular matrix production, and chemokine expression in PSC, leading to the perpetuated activation of PSC and pancreatic fibrosis [32]. A combination of short-term administration of caerulein and long-term intraperito-

neal administration of ethanol led to the activation of PSC and fibro-inflammatory responses in the pancreas [33]. Chronic ethanol consumption accelerated pancreatic fibrosis in response to caerulein-induced pancreatitis in rats [34].

Animal studies have suggested that endotoxin in the microbiota might also be involved. Gut permeability is increased in alcoholics, allowing translocation of gram-negative bacteria across the mucosal barrier and allowing bacterial endotoxins to enter the circulation. A combination of Lieber-DeCarli ethanol-enriched diet and repeated injection of lipopolysaccharide (LPS) developed acute acinar cell injury, activation of PSC and fibrosis [35]. Repeated LPS injection caused pancreatic fibrosis in ethanol-fed rats, but not in rats fed the control diet. When ethanol administration was continued, the activation of PSC and fibrosis persisted, but resolved soon after ethanol was discontinued [36]. Conversely, continued alcohol intake perpetuates pancreatic injury by inhibiting apoptosis and promoting the activation of PSC. These findings indicate the importance of abstinence to prevent the progression of acute pancreatitis to CP.

Genetic Factors Predisposing for the Development of ACP

Recent studies have identified several risk loci in ACP. The serine protease inhibitor Kazal type 1 (SPINK1), also known as pancreatic secretory trypsin inhibitor, acts as the first line of defense against prematurely activated intracellular trypsinogen by inhibiting up to 20% of trypsin activity within the pancreas. A meta-analysis of 12 studies showed the association of the *SPINK1* c.101A>G (p.N34S) variant with ACP (OR = 5.28, 95% CI: 3.45–8.09) in a Caucasian population, which was smaller than that in idiopathic CP (OR = 13.64, 95% CI: 8.86–21.00) [37]. The loss-of-function *SPINK1* c.194+2T>C (IVS3+2T>C) variant is commonly found in CP patients in east Asia. The *SPINK1* c.194+2T>C variant is overrepresented in patients with ACP (OR = 30.59, 95% CI: 16.61–56.34) [38]. The association of the variants in the chymotrypsinogen C gene (*CTRC*) with ACP has been shown. LaRusch et al. [39] reported that the synonymous *CTRC* variant c.180C>T (p.G60=) was overrepresented in CP of all etiologies, but not in recurrent acute pancreatitis as compared with controls (16.8% in CP, 11.9% in recurrent acute pancreatitis, 10.8% in controls). The *CTRC* c.180T allele was overrepresented in ACP patients (20.8%) compared to NACP patients (12.4%) (OR = 1.9, 95% CI: 1.30–2.79). A meta-analysis of four studies showed that the *CTRC* c.180T allele was overrepresented in CP (OR = 1.99, 95% CI: 1.49–2.67) [40].

A genome-wide or exome-wide approach overcomes the limitations of a candidate gene approach, enabling the discovery of new and unsuspected pancreatitis susceptibility genes. A genome-wide study from North America identified the association of common variants in the *CLDN2-MORC4* (rs7057398 and rs12688220) and *PRSS1-PRSS2* loci (rs10273639) conferred an increased risk of ACP, but not with alcohol-associated cirrhosis or alcohol dependence [41]. A meta-analysis of five studies from worldwide countries has confirmed the association of *PRSS1* rs10273639 with ACP (pooled OR = 1.67, 95% CI: 1.56–1.78) [42]. Functional studies indicated that the rs10273639 or rs4726576, which is in perfect linkage disequilibrium with rs10273639, altered the intrapancreatic trypsinogen levels [43]. Importantly, although the degree of association varies, most of the variants associated with ACP have associations with NACP, suggesting common mechanisms for alcohol-related and non-ACP. Another genome-wide study from Europe replicated previously reported risk loci *CLDN2-MORC4*, *CTRC* (c.180C>T, p.G60=), *PRSS1-PRSS2*, and *SPINK1* (c.101A>G, p.N34S) in ACP patients [44]. In addition, this study identified *CTRB1-CTRB2* (chymotrypsin B1 and B2) as a new risk locus for ACP and NACP. The association within the *CTRB1-CTRB2* locus was linked to a 16.6 kb inversion that altered CTRB1/CTRB2 expression, thereby affecting protective trypsinogen degradation. Importantly, the association of the previously reported and new risk loci was observed when compared with chronic alcoholics, suggesting that these loci are associated with the pancreas-specific injury among alcoholics.

Smoking and Chronic Pancreatitis

Recent clinical studies have shown that smoking is another important risk factor for CP, and the underlying mechanisms linking smoking and CP are being elucidated. Importantly, ethanol and smoking synergically affect the development and course of CP.

Clinical Observations

There is accumulating clinical evidence that smoking is a dose-dependent risk factor, independent of alcohol, for CP. Smoking is a risk factor for the progression from AP to recurrent AP and CP [45]. Compared to the never smoker or former smoker, current smoker had a risk of recurrent AP (OR = 2.77, 95% CI: 1.69–4.53) and CP (OR = 3.62, 95% CI: 1.98–6.60). There have been several meta-analyses that assessed the risk of CP among smokers. A meta-analysis of 12 studies showed that, compared to lifetime nonsmokers, pooled risk estimates (95% CI)

for current smokers were 2.8 (1.8–4.2) overall and 2.5 (1.3–4.6) [46]. The risk diminished significantly after smoking cessation, as the RR estimate for former smokers dropped to a value of 1.4 (1.1–1.9). A recent systematic review and meta-analysis of 22 studies revealed the summary relative risks (RR) (95% CI) for CP compared to never smokers were 3.00 (1.46–6.17) in ever, 2.72 (1.74–4.24) in current, and 1.27 (1.00–1.62) in former smokers [47]. Another meta-analysis of 10 prospective studies revealed that RR (95% CI) for CP were 1.93 (1.60–2.32) in current smokers, 1.30 (1.08–1.57) in former smokers, and 1.59 (1.39–1.82) in ever smokers compared to never smokers [48]. Dose–response analysis revealed that the summary RR per 10 pack-years was 1.22 (1.11–1.33) for CP.

Smoking accelerates the progression of CP. Smoking is independently associated with earlier onset, recurrence, appearance of calcifications, and diabetes mellitus [49]. Smoking was associated with approximately 5-year-earlier diagnosis of ACP, and with the appearance of pancreatic calcifications (hazard ratio [HR] = 4.9, 95% CI: 2.3–10.5) and diabetes (HR = 2.3, 95% CI: 1.2–4.2), independent of alcohol consumption [50]. The lower risks for CP development and progression in former smoker than those in current smoker suggest that smoking cessation decreases the risk and progression of CP. Smoking cessation would be an important strategy for primary as well as secondary prevention of pancreatitis. In addition to alcohol, physicians should routinely counsel patients for the benefits of smoking cessation. Widespread recognition of the association between smoking and CP could potentially curtail smoking rates in subjects with CP and those at risk of CP [51].

Pathophysiology

Among the more than 4000 compounds in cigarette smoke, effects of cigarette smoke, nicotine, and the tobacco-specific most abundant nitrosamine known as nicotine-derived nitrosamine ketone (NNK) have been studied alone or in combination with ethanol with regard to pancreatic diseases [52]. Major cellular components of the pancreas including pancreatic acinar cells, ductal cells, and PSC express nicotinic acetylcholine receptors, which bind to nicotine and NNK, suggesting that cigarette smoke and its major components directly affect pancreatic cells.

Cigarette smoke and its components affect cell functions and homeostasis in pancreatic cells [7, 52 and references therein] (Table 48.2). Cigarette smoke reduced pancreatic bicarbonate secretion in part by disrupting CFTR. NNK caused premature activation of trypsinogen and chymotrypsinogen in isolated pancreatic acinar cells. Nicotine activates multiple signal transduction pathways resulting in high levels of intracellular calcium release and cell injury. Clinically relevant concentrations of cigarette smoke component NKK could activate PSC, suggesting a potential mechanism for smoking-induced CP progression [53].

Table 48.2 Effects of cigarette smoke and its components on pancreatic cells [7,52].

1. induces mitochondrial damage
2. elevates intracellular calcium levels
3. disrupts expression and function of the CFTR in ductal cells
4. decreases fluid and bicarbonate secretion
5. induces endoplasmic reticulum stress
6. promotes oxidative stress
7. activates pancreatic stellate cells to promote fibrosis

CFTR: cystic fibrosis transmembrane conductance regulator.

In animal studies, rats exposed to high-dose cigarette smoke for up to 12 weeks developed a chronic inflammation resulting in pancreatic fibrosis and scarring of pancreatic acinar cell structure [54]. The ratio of trypsinogen to its endogenous trypsin inhibitor was elevated after chronic cigarette smoke exposure for 3 months, suggesting an increased vulnerability to self-digestion of the pancreas [55]. Exposure to nicotine caused the production of reactive oxygen species in pancreatic acinar cells [56]. Both nicotine and NNK have been shown to induce morphological changes in the pancreas consistent with those seen in pancreatitis. Furthermore, nicotine affects pancreatic secretion and NNK induces premature zymogen activation, two well-known features of pancreatitis. These cigarette toxins may mediate both pro- and anti-inflammatory pathways and can induce changes in pancreatic acinar cell function at the level of transcription, leading to conditions such as thiamin deficiency and mitochondrial dysfunction. Such circumstances could leave the pancreas prone to the development of pancreatitis.

Cigarette smoke might contribute to CP development through the modulation of immune cells. Xue et al. reported a role of aryl hydrocarbon receptor agonists, such as dioxin and benzo[a]pyrene, in smoking-associated CP [57]. Aryl hydrocarbon receptor ligands in cigarette smoke induces IL-22 production in $CD4^+$ T cells through aryl hydrocarbon receptors during the pancreatic damage. IL-22 interacts with IL-22 receptor on PSC and upregulates production of extracellular matrix, leading to the development of pancreatic fibrosis. The role of IL-22 was further supported by the higher serum IL-22 levels in current smokers with CP. AhR ligands did not induce fibrosis in the absence of

caerulein, suggesting that AhR activation by cigarette smoke alone is not sufficient, and additional pancreatic insults are required to induce CP.

Because most drinkers smoke, it is interesting to see the interaction between alcohol and smoking in CP. Lugea et al. [58] reported that smoking disrupts the protective adaptive mechanism that prevents ethanol-induced damage. Cigarette smoke extracts reduced spliced XBP1 levels, and increased ethanol-induced oxidative and ER stresses, leading to cell death in pancreatic acinar cells. These might be mechanisms by which alcohol and smoking interact and worsen acinar cell injury and pancreatitis.

In summary, cigarette smoke and its components affect cell functions and homeostasis in a similar manner to ethanol and its metabolites. As in the case of ethanol, cigarette smoke or its components alone did not induce CP and additional insults are required for CP. Mechanisms of synergy between alcohol and cigarette smoke have been identified. Because only a small portion of smokers develop pancreatitis, it is reasonable to assume that there might exist some genetic background of smoking-associated CP. Further studies are warranted to clarify this issue.

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