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TOPIC HIGHLIGHT

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Molecular mechanisms of alcohol associated pancreatitis

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Abstract

Alcohol abuse is commonly associated with the development of both acute and chronic pancreatitis. Despite this close association, the fact that only a small percentage of human beings who abuse alcohol develop pancreatitis indicates that alcohol abuse alone is not sufficient to initiate clinical pancreatitis. This contention is further supported by the fact that administration of ethanol to experimental animals does not cause pancreatitis. Because of these findings, it is widely believed that ethanol sensitizes the pancreas to injury and additional factors trigger the development of overt pancreatitis. How ethanol sensitizes the pancreas to pancreatitis is not entirely known. Numerous studies have demonstrated that ethanol and its metabolites have a number of deleterious effects on acinar cells. Important acinar cells properties that are affected by ethanol include: calcium signaling, secretion of zymogens, autophagy, cellular regeneration, the unfolded protein response, and mitochondrial membrane integrity. In addition to the actions of ethanol on acinar cells, it is apparent that ethanol also affects pancreatic stellate cells. Pancreatic stellate cells have a critical role in normal tissue repair and the pathologic fibrotic response. Given that ethanol and its metabolites affect so many pancreatic functions, and that all of these effects occur simultaneously, it is likely that none of these effects is "THE" effect. Instead, it is most likely that the cumulative effect of ethanol on the pancreas predisposes the organ to pancreatitis. The focus of this article is to highlight some of the important mechanisms by which ethanol alters pancreatic functions and may predispose the pancreas to disease.

Key words: Pancreatitis; Alcoholic pancreatitis; Alcoholic acute pancreatitis; Alcoholic chronic pancreatitis

Core tip: Alcohol abuse is commonly associated with the development of acute and chronic pancreatitis. Despite this close association, the fact that only a small percentage of human beings who abuse alcohol develop pancreatitis indicates that alcohol abuse alone is not sufficient to initiate clinical pancreatitis. It is widely believed that ethanol sensitizes the pancreas to injury and additional factors trigger the development of overt pancreatitis. How ethanol sensitizes the pancreas to pancreatitis in not entirely known. We will review the mechanisms by which ethanol is thought to sensitize human beings to pancreatic injury.

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INTRODUCTION

The pancreas is a complex organ, containing both exo-

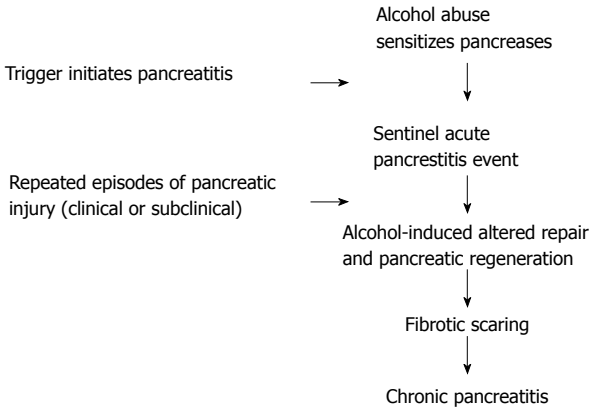

Figure 1 Proposed model for the development of alcoholic chronic pancreatitis. This proposed model incorporated alcohol abuse into the seminal acute pancreatitis event (SAPE) model proposed by Whitcomb. Alcohol metabolism results in biochemical and molecular changes in acinar cells that sensitizes the pancreas to injury. A secondary trigger initiates an initial episode of acute pancreatitis. This is the SAPE. Repeated clinical or subclinical episodes of pancreatitis coupled with ethanol-induced aberrant repair and regeneration of the damaged pancreas leads to fibrotic scarring which eventually results in chronic pancreatitis.

crine and endocrine components. The endocrine component of the pancreas comprises only about 1%-2% of the organ, and is responsible for the production of insulin and glucagon, both of which regulate glucose homeostasis. The exocrine component comprises the vast majority of the pancreas; it is composed of acinar, stellate, and ductal cells. The acinar cells produce digestive enzymes, which facilitate the digestion of carbohydrates, proteins, and lipids. The ductal cells form a network that serves as a conduit for delivery of these enzymes into the duodenum. The pancreatic stellate cells synthesize and degrade extracellular matrix proteins.

Pancreatitis, or inflammation of the pancreas, is a necroinflammatory disease of the pancreas that can manifest as either an acute or chronic disease. Acute pancreatitis is characterized by various degrees of acinar cell damage with concomitant local and systemic inflammation, mediated by inflammatory cytokines and chemokines[1]. Acute pancreatitis is usually a self-limiting condition. Unfortunately, in 10% to 20% of clinical cases, acute pancreatitis progresses to severe acute pancreatitis, a disease with high morbidity and mortality. In the United States alone there are approximately 210000 new clinical cases of acute pancreatitis a year[2]. In 2009, acute pancreatitis was the most common gastrointestinal disease requiring hospitalization. Additionally, it was estimated that acute pancreatitis accounted for more than 2.5 billion dollars in direct and indirect costs[3]. Obviously, pancreatitis is a serious public health concern.

Chronic pancreatitis is a progressive disease characterized by severe pain, persistent pancreatic inflammation, and the development of fibrotic scarring, as well as the loss of endocrine and exocrine function. It has been demonstrated in a long-term prospective study that alcoholic chronic pancreatitis normally progresses from acute pancreatitis. Additionally, this study demonstrated that the progression of acute pancreatitis to chronic pancreatitis is associated with the frequency and severity of the acute attacks[4]. These findings are supported by the observation that individuals who suffer frequent attacks of acute pancreatitis progress to chronic pancreatitis more rapidly[5]. These findings led Whitcomb to propose that a sentinel acute pancreatitis event (SAPE) is required for the development of chronic pancreatitis[6] (Figure 1). Therefore, it appears that although acute and chronic pancreatitis have different clinical manifestations, the mechanisms by which the disease process is initiated is likely similar[7]. Unfortunately, there currently is no treatment, other than palliative care, for either of these diseases.

One of the most common factors associated with both acute and chronic pancreatitis is alcohol abuse[8]. In fact, the association between alcohol abuse and pancreatic disease has been recognized for well over 100 years[9]. It has been known for sometime that the risk of developing pancreatitis increases with increasing alcohol consumption. Recent studies have shown that a threshold of approximately 5 drinks/d (60 g of ethanol) is required for significantly increased risk of developing pancreatitis[10-12]. Although numerous studies have demonstrated direct toxic effects of ethanol and its metabolites on the pancreas, the majority of heavy drinkers (even those consuming more than 5 drinks a day) do not develop pancreatitis[8,12,13]. This fact clearly indicates that alcohol abuse itself is not sufficient to cause pancreatitis, and an additional insult or additional factors are required for the development of clinical pancreatitis. Among the factors suggested to be involved in alcoholic pancreatitis are: smoking, high fat diet, obesity, genetics, and infectious agents[12-16].

Despite the long-standing recognition of the association between alcohol and pancreatitis, the biochemical and molecular processes by which ethanol influences the initiation and progression of these diseases is not well understood. It is thought that the toxic effects of ethanol and/or the by-products of ethanol metabolism sensitize the pancreas; thereby, lowering the threshold to damage from other factors. Ethanol has been shown to affect a number of pathways and functions important in acinar cells. Alteration of these pathways may individually or cumulatively sensitize the pancreas, and lower the threshold of the pancreas to the development of overt pancreatitis. Ethanol has been shown to affect a number of pathways and functions important in acinar cells (Table 1).

Both the rapid course of acute pancreatitis and the relative inaccessibility of pancreatic tissue for examination, prior to the development of fibrotic damage in chronic pancreatitis, have hampered detailed investigations using tissue from human beings. This has contributed to our limited understanding of the mechanisms that lead to the initiation and the progression of alcoholic pancreatitis. Because of this, much of our understand-

Table 1 Mechanisms by which ethanol is thought to sensitize the pancreas to pancreatitis

Alteration of cell death pathways
Altered vesicular trafficking
Impaired autophagy
Impaired tissue repair
ER stress
Mitochondrial dysfunction

ing of pancreatitis in general, and alcoholic pancreatitis in particular, has come from the use of preclinical animal models. Preclinical models used to investigate alcoholic pancreatitis normally utilize mice or rats administered ethanol. Ethanol administration to experimental animals is commonly accomplished through the Tsukamoto-French intragastric method[17], the Lieber-DeCarli pair feeding method[18], or the Cook-Meadows model of providing ethanol in the drinking water[19,20]. Pancreatic cells are either isolated from the animals administered ethanol or pancreatitis is induced. Among the more common methods of inducing pancreatitis in these animals are: bile duct ligation, treatment with supraphysiological concentrations of the cholecystokinin (CCK) analogue caerulein, or treatment with trinitrobenzene sulfonic acid (TNBS)[21]. More recently, methods designed to be more clinically relevant have been reported. These methods include chronic ethanol administration followed by treatment with gram-negative bacterial lipopolysaccharide (LPS)[22,23], or infection with Coxsackievirus CVB3[16,24,25].

Unfortunately, no animal model of chronic pancreatitis recapitulates all of the manifestations of chronic pancreatitis in human beings. It has been demonstrated that alcohol administration to rats and mice results in acinar cell loss and enhanced fibrosis in animals subjected to caerulein-induced pancreatic injury[26,27]. Therefore, these models may be useful in elucidating the mechanisms by which ethanol alters normal pancreatic repair, and predisposes the pancreas to fibrosis.

It is the focus of this article to review and highlight some of the molecular events that may adversely affect the pancreas, and sensitize the pancreas to the initiation or progression of alcoholic pancreatitis.

ETHANOL METABOLISM

Many of the deleterious effects of ethanol are attributed to the by-products produced during its metabolism. Like the hepatocytes of the liver, the pancreatic acinar cells have the ability to metabolize ethanol by both oxidative and nonoxidative pathways. The oxidative metabolism of ethanol is catalyzed by two enzymes: the cytosolic enzyme, alcohol dehydrogenase, and the microsomal enzyme, cytochrome P450 2E1. Ethanol metabolism by both of these enzymes generates acetaldehyde and reactive oxygen species. Although the pancreas expresses both alcohol dehydrogenase and cytochrome P450 2E1, the capacity for ethanol oxidation by the pancreas is significantly less than that of the liver[28,29]. Therefore, the actions of the oxidative metabolites of ethanol oxidation may result from both pancreatic metabolism and systemic metabolism of ethanol.

Nonoxidative metabolism of ethanol is carried out by a number of enzymes, the most important being the fatty acid ethyl ester synthases. Metabolism of ethanol by these enzymes generates fatty acid ethyl esters (FAEEs). The pancreas possesses high fatty acid ester synthase activity. Thus, the capacity for nonoxidative metabolism of ethanol in the pancreas is high[30]. In fact, a study of individuals who were intoxicated at the time of death revealed that the concentration of FAEEs in the pancreas was higher than any other organ analyzed[30]. Thus, because the oxidative metabolism of ethanol in the pancreas is relatively low, the nonoxidative metabolism of ethanol may be more important and the production of FAEEs, and their toxic effects, may be accentuated. Because the by-products of ethanol metabolism have been demonstrated to cause toxicity in other organs, a great deal of work has been performed investigating the actions of the various ethanol metabolites on the pancreas.

CELL DEATH

Cell death during an episode of acute pancreatitis can occur by one of two mechanisms: apoptosis or necrosis. The distinction between the two types of cell death not only has biological implications in the development of acute pancreatitis, but also affects the clinical presentation by influencing the severity of the illness[8]. Clinically, according to the 2012 Atlanta Classification of Acute Pancreatitis, the presence of necrosis and the number of organs affected by the subsequent inflammatory response determines the severity of acute pancreatitis (mild, moderate, severe) and dictates the short-term and long-term management of these patients[31].

While necrosis and apoptosis both lead to cell death, their respective mechanisms of achieving this end are quite different. Apoptosis, or programmed cell death, is a process by which cellular constituents are cleaved by cysteine-dependent, aspartate-directed enzymes, known as caspases. Apoptosis is mediated by caspases 3 and caspases 8. Caspase 8 is the initiator of the caspase cascade and cleaves caspase 3, which mediates many of the cellular changes that lead to apoptotic death. In pancreatitis, these caspases are activated by the release of cytochrome c from mitochondria[32]. The release of cytochrome c is caused by the depolarization of mitochondria. It appears this depolarization is a result of the opening of the mitochondrial permeability transition pore, which is caused by sustained increased calcium levels in the cytosol[33]. Ultimately, there is an organized dismantling of the cell. This leads to cell shrinkage and nuclear chromatin condensation, while preserving the integrity of the plasma membrane. Because the plasma membrane remains intact, there is very little leakage of intracellular material into the extracellular space, and therefore; there is little

activation of inflammatory cytokines.

In contrast to the organized dismantling of the cell in apoptosis, necrosis involves intracellular swelling of organelles and rupture of the plasma membrane. This results in the release of the contents of the cell into the extracellular space, which causes an inflammatory response. It has been shown in a number of preclinical animal models of pancreatitis that the severity of pancreatitis is increased with increasing necrotic cell death[8]. Additionally, perhaps the most important prognostic indicator of the severity of pancreatitis in human beings is the amount of necrosis[31].

In preclinical animal models of pancreatitis, ethanol has been shown to cause a shift in cell death from apoptosis to necrosis. This shift has been shown to occur through several mechanisms. It has been shown that the nonoxidative metabolites of ethanol, FAEEs, activate inositol trisphosphate receptors on the endoplasmic reticulum. Activation of these receptors causes release of calcium into the cytosol. As stated above, the sustained increases in cytosolic calcium results in mitochondria depolarization and loss of ATP production. Without ATP, the cells are unable to complete the apoptotic process and necrosis occurs[8].

Ethanol has also been shown to inhibit the JAK2/STAT1 pathway. Attenuated activity of this pathway leads to decreased activity of both caspase 8 and caspase 3[32]. With lower activity of these caspases, cell death by necrosis is increased while apoptotic cell death is reduced.

Ethanol also increases the pancreatic expression of cathepsin B[32]. Cathepsin B is a cysteine protease that is thought to play a major role in the intrapancreatic conversion of trypsinogen to trypsin. It has been shown that in pancreata of ethanol-fed rats, increased expression of cathepsin B result from increased levels of the transcriptional activators Ets-1 and Sp1[32]. Increases in Sp1 and Ets-1 enhance expression of cathepsin B, which leads to activation of trypsin and a shift from apoptosis to necrosis in pancreatic acinar cells[32]. These findings demonstrate that ethanol can affect the mechanism of cell death in acinar cells, and thereby influence the severity of the disease.

EFFECTS OF ETHANOL ON ZYMOGEN SECRETION

One of the primary roles of the exocrine pancreas is the synthesis and secretion of digestive enzymes. The pancreas is protected from the actions of these potentially dangerous enzymes because they are synthesized as inactive zymogens and packaged into exocytotic vacuoles, known as zymogen granules. Although ethanol has many effects on acinar cells that contribute to the development of pancreatitis, the inappropriate activation of zymogens is likely a critical component of this pathologic process.

Activation of trypsinogen is generally considered a pivotal event in the initiation of pancreatitis[34]. It has been reported by Gorlelich that treatment of isolated acinar cells with intoxicating concentrations of ethanol (25 mmol/L) sensitizes acinar cells to damage by causing the activation of zymogens[35]. The activation of these zymogens required an increase in cytosolic calcium and appeared to involve a low pH compartment (acid granular compartment).

Local cytosolic spikes of calcium in the apical region of acinar cells control the exocytotic secretion of zymogens. These spikes are generated by release of small quantities of calcium from internal stores[36]. In contrast, prolonged, global elevation of calcium results in the formation of empty looking zymogen granules, this is thought to be the site where trypsin is activated. In acinar cells treated with the FAEE palmitoleic acid ethyl ester, calcium was released from both the endoplasmic reticulum (the major calcium storage compartment of the cell) and the acid granular compartment, located near the apical surface. Additionally, it was demonstrated that the calcium release was primarily mediated by type 2 and 3 inositol 1,4,5 trisphosphate receptors[37].

Normally, zymogens are released from acinar cells by fusion of zymogen granules with the apical membrane. This fusion results in their release into the ducts, where they are transported to the duodenum and activated. The components absolutely required for membrane fusion consist of: SNAREs (soluble NSF [N-ethylmaleimide-sensitive fusion proteins] attachment proteins receptors) located on the target membrane, t-SNARES, and v-SNAREs, also known as vesicle-associated membrane proteins (VAMPs), located on the membrane of the vesicle. The t-SNARES syntaxin and synaptosome-associated proteins (SNAPs), form a SNARE complex that binds to its cognate v-SNARE; thus, juxtaposing the two membranes and facilitating the fusion of the membranes.

Interestingly, it has been demonstrated both *in vivo* and *in vitro,* that supramaximal treatment with cholecystokinin (CCK) causes basolateral exocytosis of zymogen granules in acinar cells[38]. Additionally, in both ethanol-fed rats or isolated acinar cells treated with physiologic concentrations of ethanol (20 mmol/L), stimulation with submaximal concentration of CCK or carbachol resulted in the exocytosis being redirected from the apical surface, where zymogens are normally secreted, to the basolateral surface[39]. The authors postulate that the ensuing ectopic activation of the zymogens in the interstitial space results in pancreatitis[39]. More detailed investigations demonstrated that this inappropriate exocytosis was mediated by phosphorylation of mammalian uncoordinated-18c (Munc 18c) by protein kinase C-alpha (PKC-α). Phosphorylation of Munc-18c results in its release from syntaxin-4, which is located on the basolateral surface of acinar cells. Syntaxin-4 is then able to complex with SNAP-23 and VAMP-8, located on the zymogen granules, to form the SNARE complex, which mediates the inappropriate basolateral exocytosis of zymogens[40]. Importantly, basolateral exocytosis has been

observed in tissue samples from a patient suffering from chronic alcoholic pancreatitis[41].

IMPAIRMENT OF AUTOPHAGY

Autophagy is a cellular process in which unnecessary or damaged cellular components or organelles are sequestered in vacuoles and transported to the lysosomes. Upon fusion with the lysosomes, the contents of the autophagic vacuoles, the autophagosomes, are degraded. Not only does this process perform an important role in ridding cells of unneeded components, but during times of low nutrient availability autophagy can provide the cell with needed constituents.

Impaired autophagy has been implicated in the pathogenesis of many diseases, including pancreatitis[15,42-45]. Importantly, it has been shown that ethanol can alter the process of autophagy in a number of organs, including the pancreas[43,46,47].

One of the histological hallmarks of pancreatitis is the accumulation of large vacuoles within acinar cells[48]. In a number of preclinical animal models of pancreatitis, as well as in tissue from a patient with acute pancreatitis, it has been demonstrated that these vacuoles are in fact autophagic vacuoles[44,45]. Further investigation revealed that these vacuoles possessed markers of both autophagosomes and lysosomes, and contained undegraded or partially degraded cellular material[45]. These findings indicate that at least the very late events in the autophagic process, namely the degradation of the components of the autolysosomes, are impaired during pancreatitis[45]. Thus, autophagy is activated during pancreatitis, and it appears that the impairment in the ability to complete this process is responsible for the vacuolization characteristic of this disease.

As mentioned above, trypsin activation is thought to be an early event in the initiation of pancreatitis. How this activation occurs is not well understood. It is generally thought that cathepsin B, is mis-sorted to the zymogens granules, where it co-localizes with trypsinogen. Subsequent cleavage of trypsinogen by cathepsin B results in the production of active trypsin. How trypsinogen and cathepsin B come in contact has always been a mystery. It now appears that the impairment in the completion of the autophagy may have a role in the co-mingling of these two enzymes.

Cathepsin L is an enzyme that degrades trypsinogen and trypsin, and cathepsin B is an enzyme that cleaves trypsinogen forming active trypsin. The two are important lysosomal hydrolases. During pancreatitis, increased levels of these enzymes are found in the zymogen granule fraction. Additionally, in alcoholic pancreatitis, as well as other forms of acute pancreatitis, the processing and activation of cathepsin L and cathepsin B is impaired[45,49]. Furthermore, it appears that the impairment in cathepsin L activity is more severe than the impairment in cathepsin B activity, particularly in the zymogen granule fraction[45]. Importantly, zymogen granules were detected in the autophagosomes/autoloysosomes. The authors propose that it is in these autophagosomes/autoloysosomes that trypsinogen and cathepsin B come in contact[45]. The imbalance between cathepsin B and cathepsin L activity in these vacuoles would favor the activation of trypsin, and the initiation of pancreatitis. Thus, impairment in the completion of the autophagic process and subsequent increase in autolysosomes may contribute not only to the accumulation of vacuoles, but also to the inappropriate intracellular activation of trypsin and the initiation of pancreatitis.

Ethanol has been shown to impair other aspects of autophagy. Using a model of alcoholic pancreatitis in which rats were chronically fed ethanol and then treated with LPS to induce acute pancreatitis, Fortunato *et al*[43] demonstrated that in the pancreata of these animals fusion of autophagosomes with the lysosome was impaired. Additional studies demonstrated that Lamp-2, a lysosomal membrane protein required for the fusion of autophagosomes with lysosomes, was depleted in the pancreata of rats suffering from alcoholic pancreatitis[43,50]. Furthermore, analysis of pancreata from human beings revealed that Lamp-2 was also decreased in the pancreata of patients suffering from chronic alcoholic pancreatitis. These results indicate that the ethanol-mediated reduction in lysosomal proteins, particularly Lamp-2, and subsequent impairment in autophagy may be a contributing factor to alcoholic pancreatitis in human beings. Although not investigated, the authors speculated that disruption in the autophagic pathway may contribute to bioenergenic failure in mitochondria. Lack of mitochondrial ATP would favor necrosis, as opposed to apoptosis. Necrotic cell death would cause inflammation and lead to the initiation of pancreatitis[43].

MITOCHONDRIAL DYSFUNCTION

Pancreatic acinar cells are among the most synthetically active cells in the body[51]. This synthetic activity requires a great deal of energy. Because of this, acinar cells contain an inordinate number of mitochondria. Thus, the actions of toxins, such as ethanol, that affect mitochondria can dramatically affect acinar cells.

Normally, acetylcholine or cholecystokinin bind to G-protein linked receptors that are located on the plasma membrane of acinar cells and stimulate the production of secondary messengers. The secondary messengers bind to inositol trisphosphate or ryanodine receptors located on the endoplasmic reticulum, zymogen granules, and endo-lysosomes. This binding results in the transient release of free calcium. Mitochondria take up this calcium, which results in their activation, the synthesis of ATP, and the secretion of zymogens.

Aberrant calcium signaling has long been considered an important factor in the initiation of pancreatic injury[52]. Pathological calcium signaling in acinar cells results from prolonged global release of calcium from the endoplasmic reticulum, as well as zymogen granules and

endo-lysosomes. In fact, early acinar cell injury (vacuolization, trypsin activation, and basolateral zymogen secretion) does not occur without prolonged, sustained release of calcium[53].

Both nonoxidative and oxidative metabolism of ethanol has been shown to contribute to mitochondrial dysfunction and acinar cell death. FAEEs, the nonoxidative metabolites of ethanol, have been shown to cause pancreatic injury by affecting calcium signaling in acinar cells[54,55]. FAEEs increase the intracellular concentration of calcium to toxic levels. This calcium increase is mediated by activation of the inositol trisphosphate receptors located on the endoplasmic reticulum, and results in global sustained increase in intracellular calcium, which causes mitochondrial membrane permeability. Mitochondrial membrane permeability can lead to cell death by either apoptosis or necrosis[56,57].

Mitochondrial membrane permeability results from opening of the mitochondrial permeability transition pore. The mitochondrial permeability transition pore is thought to have at least three major components, the voltage dependent anion channel (VDAC) located on the outer mitochondrial membrane, adenine nucleotide translocase (ANT) located in the inner mitochondrial membrane and cyclophlin-D located within the mitochondrial matrix[53].

One of the important consequences of the opening of the mitochondrial permeability transition pore and mitochondrial membrane permeability can be loss of the mitochondrial membrane potential ($\Delta\Psi M$). Loss of the $\Delta\Psi M$ results in the decreased ability of TP.

Depleted levels of ATP exacerbate the cells ability to regulate calcium by inhibiting the activity of the important ATP-dependent calcium pumps, the sacroplasmic/endoplasmic reticular calcium ATPase (SERCA) located on the ER, and the plasma membrane calcium ATPase (PMCA) located on the plasma membrane. Thus, mitochondrial membrane permeability can exacerbate the dysregulation of calcium homeostasis and lead to acinar cell necrosis.

The oxidative metabolism of ethanol also has deleterious effects on pancreatic mitochondria. Oxidative metabolism of ethanol by alcohol dehydrogenase requires oxidized nicotinamide adenine dinucleotide (NAD^+) as a cofactor, and results in the production of acetaldehyde and reduced nicotinamide adenine dinucleotide (NADH)[58,59]. Acetaldehyde is then metabolized to acetate, primarily by the mitochondrial enzyme aldehyde dehydrogenase-2. Importantly, this reaction also requires NAD^+ as a cofactor, and also results in the production of NADH[58,59]. Thus, metabolism of acetaldehyde to acetate further depletes the availability of NAD^+.

Using isolated acinar cells treated with ethanol, Shalbueva *et al*[60] demonstrated that ethanol treatment led to a decrease in the NAD^+/NADH ratio. This reduction in NAD^+ resulted in activation of the mitochondrial permeability transition pore, mitochondrial depolarization, ATP depletion, and eventually cellular necrosis[60]. Furthermore, their studies revealed that the ethanol oxidation-mediated polarization of pancreatic mitochondria was attenuated in acinar cells isolated from mice deficient in cyclophilin-D. These results indicate a role for cyclophilin-D in this ethanol metabolism-mediated mitochondrial dysfunction.

Interestingly, it has been shown in mitochondria isolated from the liver that ethanol metabolism sensitizes the mitochondrial permeability transition pore to open, in part, through increased cyclophilin-D activity and increased association of cyclophilin-D with ANT[61]. This increased activity is associated with hyperacetylation of cyclophilin-D. Acetylation of cyclophilin-D is regulated by sirtuin-3, a NAD^+-dependent deacetylase localized in the mitochondrial matrix[62]. The ethanol oxidation-mediated decrease in NAD^+ leads to decreased sirtuin-3 activity and the hyperacetylation of cyclophilin-D. Hyperacetylation of cyclophilin-D results in increased cyclophilin-D activity, increased binding to ANT, and mitochondrial permeability transition pore induction[61]. Thus, it is tempting to speculate that the ethanol oxidation-mediated induction of the mitochondrial permeability transition pore in pancreatic mitochondria is mediated by a similar NAD^+-sirtuin-3-cyclophilin-D axis.

ENDOPLASMIC RETICULUM STRESS AND THE UNFOLDED PROTEIN RESPONSE

Acinar cells are responsible for the production and secretion of large quantities of digestive enzymes. Because of this, in addition to large numbers of mitochondria, acinar cells possess an extensive endoplasmic reticulum network. The endoplasmic reticulum is the major storage site of calcium in the cell, and is the cellular organelle where the proper folding and trafficking of secretory proteins is determined. Endoplasmic reticulum stress resulting from excessive accumulation of proteins, calcium imbalance, oxidative stress, or accumulation of damaged or misfolded protein leads to a response known as the unfolded protein response (UPR)[63].

One hallmark of the UPR is the activation of the IRE1/XBP1 pathway. Inositol-requiring transmembrane kinase/endonuclease 1 (IRE1) splices X-box binding protein 1 (XBP1) messenger RNA, resulting in spliced XBP1. Spliced XBP1 is a transcriptional activator that regulates a number of genes, which encode proteins that act as ER chaperones, are involved in the proper folding of proteins, or are involved in the degradation of damaged or misfolded proteins.

The UPR is activated by pancreatic injury[64]. Additionally, it has been shown that the UPR is activated in acinar cells by long-term ethanol administration to mice[65]. Ethanol mediated UPR was characterized by increased expression of IRE1 and spliced XBP1. Although the UPR was activated, ethanol administration alone did not result in histopathologic changes to the pancreas. In contrast, administration of ethanol to mice with diminished XBP1 expression ($XBP1^{+/-}$ mice) resulted in

acinar vacuolization, cell necrosis, and inflammation[65]. The presence of pathologic changes in the pancreata of XBP1$^{+/-}$ mice led the authors to suggest that the UPR is a protective mechanism in acinar cells during endoplasmic reticulum stress. Exceeding the capacity of the UPR to compensate for endoplasmic reticulum stress results in overt pancreatitis. Thus, if the protective capacity of the UPR is exceeded, this pathway may contribute to the induction and progression of pancreatitis.

THE ROLE OF STELLATE CELLS IN ALCOHOLIC PANCREATITIS

The pancreas, like the liver, has a population of vitamin A storing cells known as stellate cells. Pancreatic stellate cells are periacinar cells located in interacinar and interlobular areas of the pancreas[66,67]. These cells are responsible for the synthesis of extracellular matrix proteins, as well as matrix metalloproteinases (enzymes that degrade extracellular matrix proteins). Thus, it appears that in the healthy organ, pancreatic stellate cells function to maintain the architecture of the pancreas by regulating the deposition and degradation of extracellular matrix components[68]. In response to pancreatic injury, pancreatic stellate cells are activated and transform into myofibroblast-like cells. Activated pancreatic stellate cells synthesize excessive amounts of extracellular matrix proteins. The accumulation of these proteins results in fibrosis. Thus, pancreatic stellate cells are intimately involved in the regulation of both normal and pathologic aspects of the pancreatitis[68,69].

Pancreatic stellate cells of both rat and human origin have the ability to metabolize ethanol through the oxidative pathway[70,71]. Rat pancreatic stellate cells possess alcohol dehydrogenase, the activity of this enzyme is induced when cells are exposed to ethanol concentrations routinely found in the blood of inebriated individuals[70]. Recently, it has also been reported that quiescent pancreatic stellate cells in human beings possess alcohol dehydrogenase activity. Additionally, this activity appeared to be upregulated in pancreatic stellate cells of individuals suffering from chronic pancreatitis and pancreatic cancer[71].

The fact that pancreatic stellate cells possess alcohol dehydrogenase activity may contribute to the development of alcoholic pancreatitis. Pancreatic stellate cells are activated when exposed to concentrations of ethanol detected in the blood of inebriated individuals (10-50 mmol/L)[70,72]. Additionally, pancreatic stellate cells isolated from both rats and human beings are activated by acetaldehyde. Ethanol and acetaldehyde not only activate pancreatic stellate cells, but also elicit responses that may have important biological consequences. Both ethanol and acetaldehyde have been shown to induce the secretion of matrix metalloproteinases in pancreatic stellate cells[73]. Furthermore, treatment of pancreatic stellate cells with ethanol induces the synthesis of interleukin-8 and connective tissue growth factor (CTGF)[72,74]. It has been suggested that these factors act in an autocrine manner to perpetuate the activation of pancreatic stellate cells[13]. This finding may help to explain both the apparent inability of the pancreas to fully recover from injury in the continued presence of ethanol, and the extremely common association between alcohol abuse and chronic pancreatitis.

Although it is well established that pancreatic stellate cells are primarily responsible for the deposition and degradation of components of the extracellular matrix, it appears that acinar cells exposed to ethanol may also contribute to the increase in extracellular matrix deposition. It has been shown that FAEEs can increase the levels of extracellular matrix proteins by inhibiting the acinar cell activity of plasmin and urokinase-type plasminogen activator (uPA) proteins involved in the degradation of the extracellular matrix components[75].

THE ROLE OF THE INFLAMMATORY RESPONSE

Inflammation mediated by cytokines, chemokines, and adhesion molecules is involved in the development of pancreatitis[1,76,77]. Interestingly, it appears that ethanol and its metabolites have a differential effect on the expression of molecules that regulate the inflammatory response. It has been shown that treatment of isolated acini with ethanol or acetaldehyde decreased the activity of two important transcriptional activators involved in the inflammatory response, specifically nuclear factor-κB (NF-κB) and activator protein 1 (AP-1). Conversely, treatment of acini with FAEEs increased the activation of these regulators of the inflammatory response[78].

The activity of NF-κB is also reduced in the pancreata of animals chronically fed ethanol[79]. However, it was demonstrated that induction of pancreatitis in rats chronically administered ethanol resulted in increased NF-κB activity, as well as increases in the mRNA levels of a number of proinflammatory cytokines, including: tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), macrophage inflammatory protein-1 (MIP-1), and monocyte chemotactic protein-1 (MCP-1)[79]. These results led the authors to suggest that the *in vitro* and *in vivo* down-regulation of these factors by ethanol reflected a protective mechanism to prevent the development of alcohol-induced pancreas[78,79].

The role of the inflammatory response in chronic alcoholic pancreatitis has also been investigated[80]. Focusing on the resident mononuclear cells of the pancreas, Deng *et al*[80] demonstrated that chronic ethanol administration reduced the number of these cells present in the pancreas. In agreement with others, they suggested that this reduction likely reflected a general immunologic suppression in the pancreas of ethanol-fed rats, and may explain why animals chronically provided ethanol do not develop chronic pancreatitis in the absence of acute pancreatic damage[80].

Despite this immunologic suppression, when pan-

creatitis was induced by caerulein, the inflammatory response in these animals was enhanced[80]. Furthermore, following repeated caerulein-induced episodes of pancreatitis, it was shown that the expression of both pro-inflammatory cytokines such as TNF-α, MIP-1α, and RANTES (regulated on activation normal T cell expressed and secreted), as well as the anti-inflammatory cytokines tissue growth factor-β (TGF-β) and interleukin-10 (IL-10) was enhanced. The increase in cytokine expression was only observed in rats fed ethanol and subjected to repeated episodes of acute pancreatitis, and was also associated with increased activation of pancreatic stellate cells and fibrosis. These findings led the authors to suggest that ethanol acts not only to sensitize the pancreas to acute pancreatitis, but also aids in the progression of chronic pancreatitis if repeated episodes of acute pancreatitis occur[80].

EFFECTS OF ETHANOL ON PANCREATIC REPAIR

It is generally accepted that fibrosis is an aberrant repair response. It appears that in the presence of ethanol, repair of the damaged pancreas is altered or never fully completed[26,27]. This may help to explain the extremely common association between alcohol abuse and chronic pancreatitis. Because ethanol and acetaldehyde can activate stellate cells, and FAEEs inhibit the degradation of extracellular matrix proteins, it is obvious that ethanol can also influence recovery of the pancreas after damage has occurred[70,72,75].

It has been demonstrated that chronic ethanol administration also delays regeneration of the damaged pancreas[81]. This delay was associated with an ethanol-mediated decrease in the expression of important developmental factors, such as PDX-1 and PTF-1a, as well as impaired activation of the Notch signaling pathway[24]. Normal pancreatic repair requires the dedifferentiation of mature acinar cells followed by their redifferentiation[82]. Thus, ethanol-mediated alterations in the expression of these important developmental factors affect the dedifferentiation/redifferentiation of acinar cells. These alterations may dramatically influence pancreatic repair.

As mentioned above, there is a close association between alcohol abuse and chronic pancreatitis. In fact, in developed countries, alcohol abuse is associated with over 70% of the reported cases[83]. Importantly, individuals suffering from chronic pancreatitis have a 20-fold greater likelihood of developing pancreatic cancer[84], a disease with a dismal prognosis. It is thought that changes that occur in the pancreas during chronic injury are associated with, or predispose the organ to, the initiation of pancreatic neoplasia. Because one of the seminal characteristics of chronic pancreatitis is aberrant tissue repair, resulting in fibrotic scarring, and ethanol consumption alters pancreatic repair, ethanol may have an indirect role in the initiation of pancreatic cancer. Thus, the effects of ethanol on repair of the damaged pancreas may be a contributing factor in pancreatic cancer, as well as alcoholic pancreatitis.

CONCLUSION

Despite the dramatic expansion of our understanding of pancreatitis in general, and how ethanol and its metabolites affect pancreatic cells, we still have not defined the mechanism of alcoholic pancreatitis. Instead, it is evident that ethanol has a plethora of toxic affects on pancreatic cells. Because all of these effects occur simultaneously, it is likely that the cumulative effects of ethanol sensitize the pancreas to damage, and that "alcoholic pancreatitis" is a multifactorial disease. Paradoxically, despite the demonstration that ethanol has numerous toxic effects on the pancreas, data from demographic studies and preclinical animal models has firmly established that ethanol itself does not cause pancreatitis. Because ethanol does not cause pancreatitis, but only sensitizes the pancreas to disease, it appears that the pancreas has developed protective mechanisms that can partially compensate for ethanol-induced cellular damage. Some of these protective mechanisms have been identified. It is likely that additional compensatory mechanisms exist. Further defining the mechanisms of ethanol-induced pancreatic injury may help define these protective mechanisms. It is hoped that this strategy will lead to the development of therapeutic targets that will prevent or reduce the severity of alcoholic pancreatitis.

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