Ethanol consumption as inductor of pancreatitis

José A Tapia, Ginés M Salido, Antonio González

Abstract
Alcohol abuse is a major cause of pancreatitis, a condition that can manifest as both acute necroinflammation and chronic damage (acinar atrophy and fibrosis). Pancreatic acinar cells can metabolize ethanol via the oxidative pathway, which generates acetaldehyde and involves the enzymes alcohol dehydrogenase and possibly cytochrome P4502E1. Additionally, ethanol can be metabolized via a nonoxidative pathway involving fatty acid ethyl ester synthases. Metabolism of ethanol by acinar and other pancreatic cells and the consequent generation of toxic metabolites, are postulated to play an important role in the development of alcohol-related acute and chronic pancreatic injury. This current work will review some recent advances in the knowledge about ethanol actions on the exocrine pancreas and its relationship to inflammatory disease and cancer.

© 2010 Baishideng. All rights reserved.

Key words: Pancreas; Calcium; Ethanol; Reactive oxygen species; Pancreatitis
Mitogen-activated protein kinase (MAPK) family members mediate a wide variety of cellular processes in response to extracellular stimuli. Once MAPKs are activated, they phosphorylate target molecules in the cytoplasm and nucleus, resulting in the regulation of gene expression concerned with proliferation and differentiation. On the other hand, Tumour Necrosis Factor (TNF) is a multifunctional proinflammatory cytokine with effects on lipid metabolism, coagulation, insulin resistance and endothelial function. Members of the TNF Receptor (TNFR) superfamily can send both survival and death signals to cells. TNF family members play important roles in various physiological and pathological processes, including cell proliferation, differentiation, apoptosis and modulation of immune responses and induction of inflammation.

The exocrine pancreas can metabolize ethanol mainly via an oxidative pathway involving the enzymes alcohol dehydrogenase and cytochrome P4502E1, although a non-oxidative pathway involving fatty acid ethyl ester synthesis has been also proposed. Therefore, metabolism of ethanol by pancreatic acinar cells and the consequent generation of toxic metabolites are postulated to play an important role in the development of alcohol-related pancreatic injury.

ETHANOL AND CALCIUM HOMEOSTASIS

The generation of repetitive local cytosolic Ca\(^{2+}\) signals in the apical pole of the pancreatic acinar cell is the starting point for the regulation of cellular function. Nevertheless, despite being one of the initial steps involved in cellular function, global and sustained changes in [Ca\(^{2+}\)]\(_i\) that are abnormal cytosolic Ca\(^{2+}\) signals, can result in necrosis. The release of Ca\(^{2+}\) through specific channels and the inhibition of Ca\(^{2+}\) pumps in intracellular stores, followed by entry of extracellular Ca\(^{2+}\), contribute to Ca\(^{2+}\) overload. Additionally, it has been proposed that abnormally elevated [Ca\(^{2+}\)]\(_i\) is a shared phenomenon that could induce trypsin premature activation, which is a previous step that can trigger acute pancreatitis.

The actions of ethanol on Ca\(^{2+}\) homeostasis are currently under study. Its effects might be due to a direct action of ethanol on Ca\(^{2+}\) handling mechanisms or to an indirect effect, mediated by a production of ROS following ethanol metabolism or by non-oxidative metabolites of ethanol. We have shown that ethanol induces Ca\(^{2+}\) mobilization in mouse pancreatic acinar cells and that this mechanism is responsible for a ROS generation and a subsequent impairment of secretory function in this cell type.

A recent work shows that ethanol itself induces the release of Ca\(^{2+}\) from intracellular stores in the form of oscillations. This effect was observed at doses ranging from 1 to 50 mmol/L. It has been shown that 50 mmol/L is a concentration within the range of blood alcohol levels in intoxicated humans. Figure 1 shows an example of [Ca\(^{2+}\)]\(_i\) oscillations evoked by ethanol. In addition, it is possible that ethanol sensitizes the tissue to physiological agonists. Therefore, a transformation of physiologically evoked oscillations in [Ca\(^{2+}\)]\(_i\) by agonists into a single transient signal will be expected. As a consequence, ethanol leads to an increase in the total Ca\(^{2+}\) mobilization in response to the agonist.

In this sense, ethanol might present a direct action on the Ca\(^{2+}\) releasing mechanisms, whereas, on the other hand, it might be reducing the action of the pumps that extrude Ca\(^{2+}\) from the cytosol i.e. the plasma membrane Ca\(^{2+}\)- ATPase (PMCA) and the sarco-endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA). A slowing down of the activity of these pumps would lead to delayed Ca\(^{2+}\) extrusion out from the cytosol and, therefore, to an accumulation of the ion within the cytosol. This inhibition of Ca\(^{2+}\) pumping activity is probably due to the generation of ROS by ethanol metabolism, as will be discussed in the following section.

On the other hand, the actions of ethanol could be directed towards the mechanisms involved in Ca\(^{2+}\) influx. Further evidences for a cytosolic Ca\(^{2+}\) overload in the presence of ethanol come from the work by Del Castillo-Vaquero et al., who show that Ca\(^{2+}\) entry into the cells is increased in the presence of ethanol. This effect is also due to the generation of free radicals. This increased Ca\(^{2+}\) influx into the cells might be responsible for the potentiation of [Ca\(^{2+}\)]\(_i\) signals in response to physiological concentrations of cholecystokinin.

However, controversy exists and there are works that show little or no effect of ethanol on Ca\(^{2+}\) signalling. These studies propose an indirect action of ethanol on Ca\(^{2+}\) homeostasis. In this case, non-oxidative metabolites of ethanol (fatty acid ethyl esters and fatty acids) are those who evoke repetitive short-lasting [Ca\(^{2+}\)] spikes. In addition, fatty acids elicit a marked reduction in the cytosolic adenosine triphosphate (ATP) level, pointing towards the mitochondria as the putative point of action. More recently, it has been proposed that these metabolites of ethanol release Ca\(^{2+}\) from the thapsigargin-sensitive ER as well as from a bafilomycin-sensitive acidic compartment, which is localized exclusively in the apical...
Pancreatic acinar cells were challenged in the presence of the intracellular Ca\(^{2+}\) chelator ethylene glycol-bis (2-amino-5-methylphenoxy) ethane-N,N,N´,N´-tetraacetic acid (BAPTA) and in the absence of extracellular Ca\(^{2+}\). The emptying of this acidic compartment is linked to intracellular activation of digestive enzymes\[14\].

Putting all these observations together it can be seen that the actions of ethanol on pancreatic acinar cells create a situation potentially leading to a Ca\(^{2+}\) overload, a critical process in the initiation of alcohol-related acute pancreatitis.

**ETHANOL AND REACTIVE OXYGEN SPECIES**

ROS are a molecular group that can be produced in the course of different physiological processes and can react with a large variety of oxidizable cellular components. Thus, ROS have been considered as pathogenic factors in a variety of tissues and cells, including the pancreas\[23,24\]. Now there is growing evidence indicating that the exocrine pancreas is vulnerable to damage from ROS generated by ethanol metabolism\[25\].

It has been proposed that ethanol induces generation of oxygen radicals in pancreatic acinar cells\[11,26,27\]. Indeed, ethanol leads to an increase in fluorescence of CM-H\(_2\)DCFDA, a stable non-fluorescent molecule that yields a polar diol that is well retained within the cells. The diol can then be oxidized by ROS to a fluorescent form; therefore this dye has been proved to be an excellent probe for determination of ROS production. In this setup, stimulation of cells with 50 mmol/L ethanol (EtOH) led to a significant increase in ROS generation. At the end of the experiment H\(_2\)O\(_2\) (100 μmol/L) was added, as a positive control for oxidation. AU, absolute units of fluorescence.

**Figure 2** Time-course of ethanol-evoked reactive oxygen species (ROS) production in mouse pancreatic acinar cells. Pancreatic acinar cells were loaded with CM-H\(_2\)DCFDA, a stable non-fluorescent molecule that yields a polar diol that is well retained within the cells. The diol can then be oxidized by ROS to a fluorescent form; therefore this dye has been proved to be an excellent probe for determination of ROS production. In this setup, stimulation of cells with 50 mmol/L ethanol (EtOH) led to a significant increase in ROS generation. At the end of the experiment H\(_2\)O\(_2\) (100 μmol/L) was added, as a positive control for oxidation. AU, absolute units of fluorescence.

By a variety of tissues and cells, including the pancreas\[23,24\]. Thus, ROS have been considered as pathogenic factors in a variety of tissues and cells, including the pancreas\[23,24\].

Furthermore, ethanol and acetaldehyde-induced activation of MAPs was blocked by the antioxidant N-acetyl-cysteine, suggesting a role of oxidative stress in the signal transduction\[30\].

To date there are several studies that implicate MAPK pathway as a critical regulator of the effects of ethanol and its metabolite, acetaldehyde, on acinar cells. Implication of the MAPK pathway as a critical regulator of the effects of ethanol and acetaldehyde on acinar cells has been proposed\[31\]. Ethanol and acetaldehyde increased the activation of all 3 subfamilies (ERK 1/2, JNK and p38 MAPK) of the MAPK pathway. Treatment of cells with the inhibitor SB203580 abolished the ethanol- and acetaldehyde-induced increase in p38 MAPK activity\[31\]. Furthermore, ethanol- and acetaldehyde-induced activation of MAPs was blocked by the antioxidant N-acetylcysteine, suggesting a role of oxidative stress in the signal transduction\[30\].

One study suggests a potential role for these pathways in contributing to the development of alcohol-related pancreatic carcinogenesis. In this study, ethanol stimulation of cell proliferation was inhibited by inhibition of mitogen-
activated protein kinase (ERK1/2) and by blocking epidermal growth factor receptor-specific tyrosine kinase\textsuperscript{[34]}. On the other hand, the intracellular signalling mechanisms regulating ethanol-induced cellular activation include the MAPK pathway and the factors responsible for mediating cell activation include ethanol itself, its metabolite acetaldehyde, oxidant stress and cytokines released during episodes of alcohol-induced pancreatic necroinflammation\textsuperscript{[37]}.

**ETHANOL AND ENZYME SECRETION**

The premature activation of digestive proenzymes, specifically proteases, within the pancreatic acinar cell is an early and critical event during acute pancreatitis. One of the early events leading to alcoholic pancreatitis seems to be the effect of ethanol on stimulus-secretion coupling mechanisms. In pancreatic acinar cells, a number of PLC-acting secretagogues, such as acetylcholine and cholecystokinin, regulate secretion via activation of a number of kinases concomitantly with the generation of repetitive local cytosolic Ca\textsuperscript{2+} signals in the apical pole. This leads to the fusion of the secretory vesicles with the apical membrane of the acinar cell and the exocytosis of the content into the extracellular space\textsuperscript{[30]}. Classically, it is known that ethanol causes a dose-dependent inhibition of enzyme synthesis without affecting exocytosis of preformed or newly synthesized protein. This is a direct inhibitory effect of ethanol and is not mediated by its metabolic processing\textsuperscript{[39]}. However, treatment of pancreatic acini with ethanol does not induce any significant effect on amylase release at a wide range of concentrations (1-50 mmol/L)\textsuperscript{[15,39]}. These results indicate that ethanol likely lacks a direct role in secretion although it decreases the enzymatic synthesis.

In contrast, ethanol can modulate the secretagogue-induced secretion. Incubation of cells with 50 mmol/L ethanol clearly reduces amylase release stimulated by CCK-8. The inhibitory effect of ethanol on CCK-8-induced amylase secretion was abolished by dithiothreitol, a sulfhydryl reducing agent, suggesting a ROS-mediated action on ethanol effects\textsuperscript{[37]}. The effect of ethanol in modulating the secretory response to CCK-8 could be related to the ability of ethanol to modulate the inflammatory response of the pancreas to low concentrations of CCK-8 (the molecular mechanism involved will be discussed further in the next section).

Data on the effects of ethanol on pancreatitis induced by high (supramaximal) concentration of CCK-8 are contradictory, with it being reported that alcohol can worsen\textsuperscript{[48]} or produce no effect on fully developed pancreatitis\textsuperscript{[44]}. However, the effect of ethanol treatment on the ability of low doses of CCK-8 to produce pancreatic damage has been clearly demonstrated\textsuperscript{[38]}. The idea that ethanol sensitizes the pancreas to the action of low doses of the hormone agrees with the results of \textit{in vitro} experiments. Pancreatic acinar cells isolated from rats fed ethanol for 9-12 mo were found more susceptible to cerulein-induced activation of trypsinogen and chymotrypsinogen than pancreatic acini from pair-fed control rats\textsuperscript{[42,48]}. In another study, ethanol with a low dose of CCK-8 but not ethanol alone was found to generate zymogen conversion that was 6-fold higher than that caused by CCK-8 alone\textsuperscript{[44]}. In summary, all these studies show that ethanol diet sensitizes rats to the development of hormone-induced pancreatitis.

**ETHANOL AND INFLAMMATION**

Over the past several years, evidence has been accumulating on the involvement of inflammatory cytokines and chemokines in the development of pancreatitis\textsuperscript{[20,42,45,46]}. It has been reported that the levels of IL-6 and TNF-\alpha were up-regulated in pancreas from rats with experimental pancreatitis and that the blockade of these cytokines attenuates the disease\textsuperscript{[45,46]}. Furthermore, a strong correlation was observed between the IL-6 level in serum and the severity of human pancreatitis\textsuperscript{[49,50]}. It has been reported that ethanol acts to sensititize the pancreas to the deleterious effects of other stimuli such as the physiological agonist CCK-8, which then leads to an inflammatory response and pancreatitis. This effect is, in part, mediated by augmenting activation of proinflammatory factors\textsuperscript{[20,42]}. It has been shown that rat cerulein pancreatitis is associated with rapid NF-\kappaB activation and that NF-\kappaB activation mediates intrapancreatic up-regulation of IL-6\textsuperscript{[49]}. Interestingly, ethanol diet potentiates the ability of CCK-8 to activate NF-\kappaB which in turns causes an increase in the cytokine expression, suggesting that activation of NF-\kappaB can be one of the mechanisms for ethanol-induced cytokine up-regulation in the CCK-treated animals\textsuperscript{[20,42,45]}.

Another observed effect of ethanol consumption is that it alone attenuated pancreatic NF-\kappaB and decreased the expression of IL-6, iNOS and MIP-2, all of which are regulated by NF-\kappaB\textsuperscript{[42]}. Furthermore, a decrease in the levels of prostaglandin E2 has been reported and could also be involved in alcohol-induced injury in the pancreas\textsuperscript{[48]}. In summary, ethanol diet causes sensitization to CCK-8-induced activation of pancreatic NF-\kappaB and cytokine/chemokine mRNA expression, and ethanol itself causes down-regulation of NF-\kappaB activity and mRNA levels for certain cytokines and chemokines. Both mechanisms i.e. hormone sensitization and down-regulation of NF-\kappaB, cytokines and chemokines, could be involved in the development of the pro-inflammatory effect of ethanol in the pancreas.

**CONCLUSION**

Pancreatic acinar cells and other pancreatic cells can metabolize ethanol and the consequent generation of toxic metabolites are postulated to play an important role in the development of alcohol-related acute and chronic pancreatic injury. Ethanol may itself, or through its oxidative or non-oxidative metabolites, lead to Ca\textsuperscript{2+} mobilization from intracellular stores, sensitization of the tissue to Ca\textsuperscript{2+} mobilizing agonists and/or decrease the activity of Ca\textsuperscript{2+} transport mechanisms. As a consequence, ethanol leads to
cytosolic Ca\(^{2+}\) overload. Intracellular Ca\(^{2+}\) overload has been related to ROS over production which, in turn, can further increase cytosolic Ca\(^{2+}\) accumulation because oxidants impair Ca\(^{2+}\) handling by the cell. In addition, ethanol or its metabolites inhibit secretagogue-induced secretion of its enzymes, that will then accumulate within the cell. Inhibition of zymogen secretion can lead to its intracellular activation, setting the starting point for autodigestion of the gland and a consequent inflammatory process. Moreover, ethanol causes an increase in the cytokine expression in response to agonists which represents a crosstalk with inflammation pathways. On the other hand, moderate and high alcohol intake levels over a lifetime might increase cancer risk through activation of a wide variety of cellular processes in response to extracellular stimuli that can lead to tumorigenesis. The putative mechanisms of action of ethanol on pancreatic acinar cells physiology are summarised in Figure 3. In conclusion, ethanol impairs the exocrine pancreas function, creating a situation potentially leading to the development of pancreatic diseases.

REFERENCES

1. Streb H, Irvine RF, Berridge MJ, Schulz I. Release of Ca\(^{2+}\) from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature 1983; 306: 67-69
2. Putney JW Jr. A model for receptor-regulated calcium entry. Cell Calcium 1986; 7: 1-12
3. Carafoli E. Calcium pump of the plasma membrane. Physiol Rev 1991; 71: 129-153
4. González A, Camello PJ, Pariante JA, Salido GM. Free cytosolic calcium levels modify intracellular pH in rat pancreatic acini. Biochem Biophys Res Commun 1997; 230: 652-656
5. Laude AJ, Simpson AW. Compartmentalized signalling: Ca\(^{2+}\) compartments, microdomains and the many facets of Ca\(^{2+}\) signalling. FEBS J 2009; 276: 1800-1816
6. Krüger B, Albrecht E, Lerch MM. The role of intracellular calcium signalling in premature protease activation and the onset of pancreatitis. Am J Pathol 2000; 157: 43-50
7. Raraty M, Ward J, Erdemli G, Vaillant C, Neoptolemos JP, Sutton R, Petersen OH. Calcium-dependent enzyme activation and vacuole formation in the apical granular region of pancreatic acinar cells. Proc Natl Acad Sci USA 2000; 97: 13126-13131
8. Asada S, Daitoku H, Matsuzaki H, Saito T, Sudo T, Mukai H, Iwashita S, Kako K, Kishi T, Kasuya Y, Fukushima A. Mitogen-activated protein kinases, Erk and p38, phosphorylate and regulate Fos protein. Cell Signal 2007; 19: 519-527
9. Kawasaki H, Onuki R, Suyama E, Taira K. Identification of genes that function in the TNF-alpha-mediated apoptotic pathway using randomized hybrid ribozyme libraries. Nat Biotechnol 2002; 20: 376-380
10. Crisdale DN, Raraty MG, Neoptolemos JP, Tepikin AV, Petersen OH, Sutton R. Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites. Proc Natl Acad Sci USA 2004; 101: 10738-10743
11. González A, Pariante JA, Salido GM. Ethanol impairs calcium homeostasis following CCK-8 stimulation in mouse pancreatic acinar cells. Alcohol 2008; 42: 565-573
12. Petersen OH, Sutton R. Ca\(^{2+}\) signalling and pancreatitis: effects of alcohol, bile and coffee. Trends Pharmacol Sci 2006; 27: 113-120
13. Ding YX, Yang K, Chin WC. Ethanol augments elevated-[Ca\(^{2+}\)]induced trypsin activation in pancreatic acinar zymogen granules. Biochem Biophys Res Commun 2006; 350: 593-597
14. Gerasimenko JV, Lur G, Sherwood MW, Ebisui E, Tepikin AV, Mikoshiba K, Gerasimenko OV, Petersen OH. Pancreatic protease activation by alcohol metabolite depends on Ca\(^{2+}\) release via acid store IP\(_3\) receptors. Proc Natl Acad Sci USA 2009; 106: 10758-10763
15. Shah AU, Sarwar A, Orabi AI, Gautam S, Grant WM, Park AJ, Shah AU, Liu J, Mistry PK, Jain D, Husain SZ. Protease Activation during in vivo Pancreatitis is Dependent upon Calciuminurin Activation. Am J Physiol Gastrointest Liver Physiol 2009; Epub ahead of print
16. Ward JB, Petersen OH, Jenkins SA, Sutton R. Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? Lancet 1995; 346: 1016-1019
17. González A, Núñez AM, Granados MP, Pariante JA, Salido GM. Ethanol impairs CCK-8-evoked amylase secretion through Ca\(^{2+}\)-mediated ROS generation in mouse pancreatic acinar cells. Alcohol 2006; 38: 51-57
18. Fernández-Sánchez M, del Castillo-Vaquero A, Salido GM, González A. Ethanol exerts dual effects on calcium homeostasis in CCK-8-stimulated mouse pancreatic acinar cells. BMC Cell Biol 2009; 10: 77
19. Lamarche F, Gonthier B, Signorini N, Eysseric H, Barret L. Impact of ethanol and acetaldehyde on DNA and cell viability of cultured neurones. Cell Biol Toxicol 2004; 20: 361-374
20. Pandol SJ, Gukovsky I, Satoh A, Lugea A, Gukovskaya AS. Emerging concepts for the mechanism of alcoholic pancreatitis from experimental models. J Gastroenterol 2003; 38: 623-628
21. del Castillo-Vaquero A, Salido GM, González A. Increased calcium influx in the presence of ethanol in mouse pancreatic acinar cells. Int J Exp Pathol 2009; Epub ahead of print
22. Crisdale DN, Sutton R, Petersen OH. Role of Ca\(^{2+}\) in pancreatic cell death induced by alcohol metabolites. J Gastroenterol...
 Tapia JA et al. Ethanol and pancreatitis  

*Hepatol* 2006; 21 Suppl 3: S14-S17  
23 Schoenberg MH, Büchner M, Beger HG. Oxygen radicals in experimental acute pancreatitis. *Hepatogastroenterology* 1994; 41: 313-319  
24 Weber H, Roessner JP, Nebe B, Rychly J, Werner A, Schroder H, Jonas L, Leitzmann P, Schneider KP, Dummerl W. Increased cytosolic Ca²⁺ amplifies oxygen radical-induced alterations of the ultrastructure and the energy metabolism of isolated rat pancreatic acinar cells. *Digestion* 1998; 59: 175-185  
25 Palmieri VO, Grattagliano I, Palasciano G. Ethanol induces secretion of oxidized proteins by pancreatic acinar cells. *Cell Biol Toxicol* 2007; 23: 459-464  
26 Wilson JS, Apte MV. Role of alcohol metabolism in alcoholic pancreatitis. *Pancreas* 2003; 27: 311-315  
27 Wittel UA, Bachem M, Siech M. Oxygen radical production precedes alcohol-induced acute pancreatitis in rats. *Pancreas* 2003; 26: e74-e80  
28 Hu R, Wang YL, Edderkao M, Lugea A, Apte MV, Pandol SJ. Ethanol augments PDGF-induced NADPH oxidase activity and proliferation in rat pancreatic stellate cells. *Pancreatology* 2007; 7: 332-340  
29 Hemple SL, Buettner GR, O'Malley YQ, Wessels DA, Flaherty DM. Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2',7'-dichlorodihydrofluorescein diacetate, 5(and 6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate, and dihydrorhodamine 123. *Free Radic Biol Med* 1999; 27: 146-159  
30 Possel H, Noack H, Augustin W, Keilholf G, Wolf G. 2,7-Dihydrofluorescein diacetate as a fluorescent marker for peroxynitrite formation. *FEBS Lett* 1997; 416: 175-178  
31 Benedetti A, Parent ME, Siemiatycki J. Lifetime consumption of alcoholic beverages and risk of 13 types of cancer in men: results from a case-control study in Montreal. *Cancer Detect Prev* 2009; 32: 352-362  
32 Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 2009; 9: 537-549  
33 Apte M, McCarron J, Pirola R, Wilson J. Pancreatic MAP kinase pathways and acetaldehyde. *Necrosis Found Symp* 2007; 285: 200-211; discussion 211-216  
34 McCarron JA, Phillips PA, Park S, Doherty E, Pirola RC, Wilson JS, Apte MV. Pancreatic stellate cell activation by ethanol and acetaldehyde: is it mediated by the mitogen-activated protein kinase signaling pathway? *Pancreas* 2003; 27: 150-160  
35 Masamune A, Kikuta K, Satoh M, Satoh A, Shimosegawa T. Alcohol activates activator protein-1 and mitogen-activated protein kinases in rat pancreatic stellate cells. *J Pharmacol Exp Ther* 2002; 302: 36-42  
36 Askari MD, Tsao MS, Cekanova M, Schuller HM. Ethanol and the tobacco-specific carcinogen, NNK, contribute to signaling in immortalized human pancreatic duct epithelial cells. *Pancreas* 2006; 33: 53-62  
37 Apte MV, Pirola RC, Wilson JS. Battle-scared pancreas: role of alcohol and pancreatic stellate cells in pancreatic fibrosis. *J Gastroenterol Hepatol* 2006; 21 Suppl 3: S97-S101  
38 Gardner JD, Jensen RT. Receptors and cell activation associated with pancreatic enzyme secretion. *Ann Rev Physiol* 1986; 48: 103-117  
39 Chapman BA, Pattinson NR. The effect of ethanol on enzyme synthesis and secretion in isolated rat pancreatic lobules. *Biochem Pharmacol* 1987; 36: 3353-3360  
40 foilitz T, Lewandowski KB, Fernández-del Castillo C, Rattner DW, Klar E, Warshaw AL. Exocrine hyperstimulation but not pancreatic duct obstruction increases the susceptibility to alcohol-related pancreatic injury. *Arch Surg* 1994; 129: 1081-1085  
41 Korsten MA, Haber PS, Wilson JS, Lieber CS. The effect of chronic alcohol administration on cerulium-induced pancreatitis. *Int J Pancreatol* 1995; 18: 25-31  
42 Pandol SJ, Periskic S, Gukovskaya I, Zaninovic V, Jung Y, Zong Y, Solomon TE, Gukovskaya AS, Tsukamoto H. Ethanol diet increases the sensitivity of rats to pancreatitis induced by cholecystokinin octapeptide. *Gastroenterology* 1999; 117: 706-716  
43 Ponnapa BC, Marciniak R, Schneider T, Hoek JR, Rubin E. Ethanol consumption and susceptibility of the pancreas to ceruleum-induced pancreatitis. *Pancers* 1997; 14: 150-157  
44 Katz M, Carangelo R, Miller LJ, Gorelick F. Effect of ethanol on cholecystokinin-stimulated zymogen conversion in pancreatic acinar cells. *Am J Physiol* 1996; 270: G171-G175  
45 Gukovskaya AS, Pandol SJ. Cell death pathways in pancreatitis and pancreatic cancer. *Pancreatology* 2004; 4: 567-586  
46 Pandol SJ. Acute pancreatitis. *Curr Opin Gastroenterol* 2006; 22: 481-486  
47 Norman JG, Franz MG, Fink GS, Messina J, Fabri PJ, Gower WR, Carey LC. Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. *Ann Surg* 1995; 221: 625-631; discussion 631-634  
48 Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, Pandol SJ. Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis. *J Clin Invest* 1997; 100: 1853-1862  
49 Gukovsky I, Gukovskaya AS, Blinman TA, Zaninovic V, Pandol SJ. Early NF-kappaB activation is associated with hormone-induced pancreatitis. *Am J Physiol* 1998; 275: G1402-G1414  
50 Heath DJ, Cruickshank A, Gudgemon M, Jehanli A, Shenkin A, Imrie CW. Role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis. *Gut* 1993; 34: 41-45  
51 Formela LJ, Galloway SW, Kingsnorth AN. Inflammatory mediators in acute pancreatitis. *Br J Surg* 1995; 82: 6-13  
52 Siegmund E, Weber H, Kasper M, Jonas L. Role of PGF2 in the development of pancreatic injury induced by chronic alcohol feeding in rats. *Pancreatology* 2003; 3: 26-35  

S- Editor Li LF L- Editor Roemmele A E- Editor Yang C