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Chronic plus binge ethanol exposure causes more severe pancreatic injury and inflammation

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ABSTRACT

Alcohol abuse increases the risk for pancreatitis. The pattern of alcohol drinking may impact its effect. We tested a hypothesis that chronic ethanol consumption in combination with binge exposure imposes more severe damage to the pancreas. C57BL/6 mice were divided into four groups: control, chronic ethanol exposure, binge ethanol exposure and chronic plus binge ethanol exposure. For the control group, mice were fed with a liquid diet for two weeks. For the chronic ethanol exposure group, mice were fed with a liquid diet containing 5% ethanol for two weeks. In the binge ethanol exposure group, mice were treated with ethanol by gavage (5 g/kg, 25% ethanol w/v) daily for 3 days. For the chronic plus binge exposure group, mice were fed with a liquid diet containing 5% ethanol for two weeks and exposed to ethanol by gavage during the last 3 days. Chronic and binge exposure alone caused minimal pancreatic injury. However, chronic plus binge ethanol exposure induced significant apoptotic cell death. Chronic plus binge ethanol exposure altered the levels of alpha-amylase, glucose and insulin. Chronic plus binge ethanol exposure caused pancreatic inflammation which was shown by the macrophages infiltration and the increase of cytokines and chemokines. Chronic plus binge ethanol exposure increased the expression of ADH1 and CYP2E1. It also induced endoplasmic reticulum stress which was demonstrated by the unfolded protein response. In addition, chronic plus binge ethanol exposure increased protein oxidation and lipid peroxidation, indicating oxidative stress. Therefore, chronic plus binge ethanol exposure is more detrimental to the pancreas.

1. Introduction

Pancreatitis is inflammation of the pancreas. There are two forms of pancreatitis: acute and chronic pancreatitis. Acute pancreatitis (AP) is caused by rapid inflammation and characterized by local/systemic inflammation and the damage to acinar cells in the exocrine pancreas. AP usually recovers as the inflammation eases. However, approximately 20% of AP progresses to severe acute pancreatitis (SAP), a disease with high morbidity and mortality (Whitcomb, 2006; Clemens et al., 2014). AP is the most common gastrointestinal disease requiring hospitalization in the United States (Peery et al., 2012; Clemens et al., 2014). In 2009, there were 275,000 admissions for AP, accounting for a direct annual cost of $2.6 billion (Peery et al., 2012). Chronic pancreatitis (CP) is a progressive inflammatory disease leading to irreversible destruction of the pancreas. It is characterized by persistent inflammation, fibrotic scarring and impaired pancreatic function. CP is manifested by a spectrum of clinical symptoms ranging from severe pain to maldigestion and diabetes. It is generally believed that CP may be caused by repeated AP and the underlying mechanisms for AP and CP may be similar (Ammann and Muellhaupt, 1994; Clemens et al., 2014).

Excessive alcohol exposure is the major cause for both AP and CP (Yadav and Lowenfels, 2006; Fagenholz et al., 2007; Pandol and Raraty, 2007; Yadav and Lowenfels, 2013). Alcoholic pancreatitis represents 36% of all cases of AP (Schneider et al., 2010). Five percent of alcoholics develop an AP (Schneider et al., 2014). Pancreatitis is the most common alcohol-related hospital diagnosis in the United States (Yang et al., 2008). The prevalence of alcoholic pancreatitis may be much higher than the current estimation. A postmortem study showed that pancreatitis was found in up to 75% of alcoholics although clinical pancreatitis is only diagnosed in <10% of alcoholic patients (Pitchumoni et

Abbreviation: ADH, alcohol dehydrogenase; AP, acute pancreatitis; ATF6, activating transcription factor 6; BEC, blood ethanol concentration; CK-18, cytokeratin 18; CLD, control liquid diet; CLD + G, control liquid diet plus gavage; CHOP, C/EBP Homologous Protein; CP, chronic pancreatitis; CYP2E1, cytochrome P450 2E1; DNP, dinitrophenol; ELD, ethanol liquid diet; ELD + G, ethanol liquid diet plus gavage; eIF2α, Eukaryotic Initiation Factor 2α; FAEEs, fatty acid ethyl esters; HMGB1, high mobility group protein B1; HNE, 4-hydroxynonenal; PARP, poly (ADP-ribose) polymerase; PERK, protein kinase R-like endoplasmic reticulum kinase; SAP, severe acute pancreatitis; UPR, unfolded protein response; XBP1, X-box binding protein-1.

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al., 1984; Dufour and Adamson, 2003). It is suggested that alcoholic AP and CP are the same disease at different stages (Ammann et al., 1996). Notably, after a first acute episode of pancreatitis, alcoholics have a much higher risk of developing CP than non-drinkers or occasional drinkers (Rocco et al., 2014).

It has been shown that the drinking pattern affects the impact of alcohol on human health. For example, the pattern of alcohol consumption may affect the progression of alcoholic liver diseases as well as the severity of damage to other organs (Bellentani et al., 1997; Stranges et al., 2004; Bertola et al., 2013). It was suggested that chronic plus binge ethanol exposure caused more severe liver damage in mice (Ki et al., 2010). Here we used a mouse model of chronic plus binge ethanol exposure to study alcohol-induced pancreatic injury. The model is similar to the drinking patterns of many alcoholics who have a background of drinking for many years (chronic) and a history of recent excessive alcohol consumption (binge). We show here that chronic plus binge ethanol exposure caused a spectrum of pancreatic injury and inflammation. It also induced endoplasmic reticulum stress and oxidative stress, therefore offering a good model to investigate how drinking patterns impact the development of pancreatitis.

2. Materials and methods

2.1. Materials

Reagents for the analysis of ethanol and glucose concentration were obtained from Analox instruments (London, UK). Rabbit anti-α-amylase, mouse anti-insulin, mouse anti-glucagon and mouse anti-tubulin antibodies were obtained from Sigma-Aldrich (St. Louis, MO). Mouse anti-M30 CytoDEATH (caspase-cleaved product of cytokeratin 18, CK18) was obtained from Roche Life Science (Mannheim, Germany). Rabbit anti-ATF6 antibody was purchased from LifeSpan Biosciences (Seattle, WA). Rabbit anti-p-eIF2α, rabbit anti-p-PERK, rabbit anti-cleaved caspase-3, rabbit anti-PARP, mouse anti-caspase 8, rabbit anti-HMGB1, mouse anti-caspase 9, and rabbit anti-Dinitrophenol (DNP) antibodies were obtained from Cell Signaling Technology (Danvers, MA). Rabbit anti-GRP78 antibody was obtained from Novus Biologicals (Littleton, CO). Rat anti-GRP94 antibody was obtained from Enzo Life Sciences (Farmingdale, NY). Rabbit anti-XBP-1 antibody was obtained from BioLegend (San Diego, CA). Mouse anti-CHOP antibody was obtained from Thermo Fisher Scientific (Rockford, IL). Rabbit anti-4-Hydroxynonenal (HNE) antibody was obtained from LifeSpan BioSciences (Seattle, WA). Rat anti-CD68 and rabbit anti-MCP-1 antibodies were obtained from AbD Serotec (Oxford, UK). Rabbit anti-CCR2 antibody was obtained from BioVision (Milpitas, CA). Mouse anti-caspase 12, rabbit anti-IL-1beta, rabbit anti-IL-6 antibodies and Amylase Assay kit were obtained from Abcam (Cambridge, MA). Antibodies directed against alcohol dehydrogenases 1 (ADH1) and cytochrome P450 2E1 (CYP2E1) were obtained from Cell Signaling Tech (Danvers, MA) and Proteintech (Rosemont, IL), respectively. In Situ Cell Death Detection kit, POD was obtained from Roche Diagnostics (Indianapolis, IN). Mouse Insulin ELISA kit and glucagon ELISA kit were from Mercodia (Uppsala, Sweden). HRP-conjugated anti-rabbit, anti-mouse, anti-goat and anti-rat secondary antibodies were purchased from GE Healthcare Life Sciences (Piscataway, NJ). Mounting media with DAPI were obtained from Vector Laboratories (Burlingame, CA). Alexa-488 conjugated anti-rabbit and Alexa-594 conjugated anti-rat antibodies were obtained from Life Technologies (Grand Island, NY). Ketamine/xylazine was obtained from Butler Schein Animal Health (Dublin, OH). Other chemicals and reagents were purchased either from Sigma-Aldrich or Life Technologies (Frederick, MD). Liquid diet was obtained from Bio-Serv (Flemington, NJ).

2.2. Animal model

Male C57BL/6 mice (8 weeks old) were obtained from Jackson Laboratories (Bar Harbor, Maine) and maintained in the Division of Laboratory Animal Resources of the University of Kentucky Medical Center. All procedures were performed in accordance with the guidelines set by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. Animals were maintained in a 12 hour/12 hour light/dark cycle with a temperature of 22 ± 1 °C and relative humidity of 60 ± 5%, and received standard chow and water ad libitum. After one week of acclimation, mice were divided into four groups: control, chronic exposure, binge exposure and chronic plus binge exposure (Fig. 1). For control group, mice were fed with a control liquid diet (CLD) for two weeks. For chronic exposure group, mice were fed with an ethanol liquid diet (ELD) containing 5% ethanol for two weeks. The Lieber-DeCarli '82 liquid diet (Control: F1259SP; Ethanol: F1258SP) was used in this study (Bio-Serv, Flemington, NJ). For binge exposure group, mice were treated with ethanol by gavage (5 g/kg, 25% ethanol w/v) daily during the last three days (CLD + G). For chronic plus binge exposure group, mice were fed with an ethanol liquid diet containing 5% ethanol for two weeks and exposed to ethanol by gavage during last three days (ELD + G). For each group, there were eight mice (n = 8). The gavage was performed at 10:00 am. Six hours after final ethanol treatment, mice were euthanized and the pancreas was dissected and processed for histological and biochemical analyses.

2.3. Determination of blood ethanol and glucose concentrations

One hour following gavage on day 1 and day 3, mice were anesthetized by intraperitoneal (IP) injection of ketamine/xylazine and blood samples were taken via the retro-orbital sinus using heparinized capillary tubes. The plasma was obtained by centrifugation and 10 μl was used to measure blood ethanol and glucose concentration using an Analox AM 1 analyzer (Lunenburg, MA) as previously described (Xu et al., 2016). The blood ethanol concentration (BEC) on day 1 was 93 ± 69 mg/dl in ELD, 368 ± 75 mg/dl in CLD + G and 406 ± 76 mg/dl in ELD + G. The BEC on day 3 was 82 ± 43 mg/dl in ELD, 428 ± 84 mg/dl in CLD + G and 450 ± 85 mg/dl in ELD + G.

2.4. Measurement of plasma insulin, glucagon and amylase

Six hours after final ethanol exposure, the blood was obtained, and the plasma was separated and stored at −80 °C for ELISA. The α-amylase activity of plasma was assessed using the amylase assay kit from Abcam (Cambridge, UK) in accordance with the manufacturer's instructions. 25 μl of the plasma sample was diluted to 50 μl for each test, and the activity of α-amylase was showed as mU/ml (nmol/min/ml). The plasma insulin and glucagon levels were detected by an insulin and glucagon ELISA Kit obtained from Mercodia (Uppsala, Sweden) according to the manufacturer's description.

2.5. Tissue preparation and immunoblotting

Animals were anesthetized with intraperitoneal injection of ketamine/xylazine (100 mg/kg/10 mg/kg), and the pancreas was dissected and immediately frozen in dry ice and then stored in −80 °C. The protein was extracted and subjected to immunoblotting analysis as previously described (Wang et al., 2015). Briefly, tissues were homogenized in an ice cold lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EGTA, 0.5% NP-40, 0.25% SDS, 1 mM PMSF, 5 μg/ml leupeptin, and 5 μg/ml aprotinin. Homogenates were centrifuged at 20,000*g* for 30 min at 4 °C and the supernatant fraction was collected. After determining protein concentration, aliquots of the protein samples (30 μg) were separated on a SDS-polyacrylamide gel by electrophoresis. The separated proteins were transferred to nitrocellulose membranes. The membranes were blocked with either 5% BSA in 0.01 M PBS (pH 7.4) and 0.05% Tween-20 (TPBS) at room temperature for 1 h. Subsequently, the membranes were probed with primary