Palmitic acid aggravates inflammation of pancreatic acinar cells by enhancing unfolded protein response induced CCAAT-enhancer-binding protein β–CCAAT-enhancer-binding protein α activation

Jianghong Wu [1], Guoyong Hu [1], Yingying Lu [1], Junyuan Zheng, Jing Chen, Xingpeng Wang *, Yue Zeng *

Department of Gastroenterology, Shanghai First People's Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, China

ABSTRACT

Hypertriglyceridemia is an independent risk factor for acute pancreatitis, in which the pathological mechanisms are not fully illustrated. Intracellular inflammatory response is a key pathological response in acute pancreatitis and endoplasmic reticulum stress has been suggested to induce inflammation and CCAAT-enhancer-binding protein expression. Therefore, the current study aims to elucidate the possible relationship between endoplasmic reticulum stress and inflammation in hypertriglyceridemia associated pancreatitis and the possible involvement of CCAAT-enhancer-binding protein. In cholecystokinin-8 stimulated rat primary acinar cells, incubation with palmitic acid caused the activation of endoplasmic reticulum stress and inflammatory responses. Pre-incubation with the chemical chaperone 4-phenylbutyric acid inhibited inflammatory responses induced by palmitic acid, whereas stimulation with the endoplasmic reticulum stress inducer thapsigargin alone induced inflammatory responses. Meanwhile we found that the transcription factors CCAAT-enhancer-binding protein α and CCAAT-enhancer-binding protein β were also induced in the palmitic acid-stimulated pancreatic acinar cells, and were similarly inhibited by 4-phenylbutyric acid pre-incubation and induced by thapsigargin stimulation alone, indicating that endoplasmic reticulum stress was responsible for CCAAT-enhancer-binding protein α and CCAAT-enhancer-binding protein β induction in the pancreatic acinar cells. Knockdown of CCAAT-enhancer-binding protein β by siRNA transfection inhibited inflammatory responses and CCAAT-enhancer-binding protein α induction but did not affect endoplasmic reticulum stress. Our study provides strong evidence that in response to palmitic acid stimulation, endoplasmic reticulum stress induces inflammatory responses in pancreatic acinar cells through induction of the CCAAT-enhancer-binding protein family, wherein CCAAT-enhancer-binding protein β activation is responsible for CCAAT-enhancer-binding protein α activation.

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1. Introduction

Acute pancreatitis (AP) is a sudden inflammatory disease of the pancreas, in which intracellular inflammatory response is a key pathological response. Hypertriglyceridemia (HTG) is an independent risk factor for acute pancreatitis and tends to lead to a more severe form of pancreatitis (Valdivielso et al., 2014). It is commonly accepted that free fatty acids released from excess triglycerides (TG) hydrolyzed by high levels of pancreatic lipase can damage pancreatic acinar cells (PACs) (Durgampudi et al., 2014; Noel et al., 2016). However, the mechanism by which HTG aggravates AP is poorly understood, which causes major problems in clinical treatment of the disease (Valdivielso et al., 2014). ER stress activation

has also been observed in all models of experimentally induced pancreatitis and inhibition of ER stress with chemical chaperones, including 4-phenylbutyric acid (4-PBA) and tauroursodeoxycholic acid, has shown protective effects against pancreatic injury (Malo et al., 2013; Malo et al., 2010). Our previous *in vivo* results have shown that endoplasmic reticulum (ER) stress occurs in PACs in HTG-aggravated AP (Zeng et al., 2012).

A major feature of the ER stress response is the unfolded protein response (UPR), which relies on a highly coordinated response involving three parallel signaling branches using the transmembrane proteins protein kinase–like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring ER-to-nucleus signal kinase 1 (IRE1) (Bernales et al., 2006). In response to ER stress, these three transmembrane proteins are activated, in which activation of PERK pathway leads to phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), ATF6 (an active transcription factor) activation leads to expression of target genes including ER chaperone glucose-related peptide 78 (GRP78), and IRE1 activation catalyzes removal of a small intron from X-box–binding protein 1 (XBP1) mRNA (Danino et al., 2015).

ER stress and the UPR have recently been linked to inflammation in a variety of human pathologies including autoimmune diseases, infection, neurodegenerative disease, and metabolic disorders. All three signaling pathways of UPR have been shown to induce inflammation through nuclear factor-kappa B (NF-κB) activation through different mechanisms (Hummasti and Hotamisligil, 2010). The crosstalk between inflammation and ER stress has been suggested to play a significant role in pancreatic β cell dysfunction (Oslowski et al., 2012; Zhang and Kaufman, 2008). However, the interaction between ER stress and inflammation in HTG-aggravated AP and acinar cell injury remains elusive. Further understanding of those issues may enable the development of novel therapies of HTG-aggravated AP.

The CCAAT enhancer-binding protein (C/EBP) family of basic leucine-zipper transcription factors includes C/EBPα, -β, -γ, -δ, and -ε, and the ER stress gene C/EBP homology protein (CHOP), which must heterodimerize with other members of this family, most notably C/EBPβ, in order to function (Rahman et al., 2012; Ron and Habener, 1992). C/EBPβ performs diverse functions, participating in the regulation of genes that contribute to the acute phase response, glucose metabolism, tissue differentiation and inflammation (Matsuda et al., 2010; Matsuda et al., 2015; Ramji and Foka, 2002). Previous studies have shown that C/EBPβ expression is upregulated by stimuli that induce ERstress, such as exposure to thapsigargin, and inhibition of C/EBPβ expression reduced ER stress-associated cell failure and diseases (Matsuda et al., 2010; Matsuda et al., 2015). These findings suggest that C/EBPβ is central to the pathogenesis of metabolism-associated diseases. While C/EBPβ is a key regulator of inflammation, its pivotal role in the pathogenesis of AP remains relatively unexplored. This study was conducted to illustrate the relationship between ER stress and inflammation in HTG-aggravated AP and to identify the underlying mechanism.

2. Material and methods

2.1. Materials

Caerulein, palmitic acid (PA), cholecystokinin-8 (CCK-8), 4-phenylbutyric acid (4-PBA) and thapsigargin were purchased from Sigma-Aldrich Chemical (MO, USA). Bovine serum albumin (BSA) was purchased from Roche (Basel, Switzerland). Antibodies against sXBP-1 (#sc-7160), C/EBPα (#sc-61), C/EBPβ (#sc-150) and β-actin (#sc-81178) were from Santa Cruz Biotechnology (TX, USA). Antibodies against IκBα (#4812), IκBβ (#9248), CHOP (#2895) and phosphorylated eIF2α (#3398) were from Cell Signaling Technology (MA, USA). Antibodies against NF-κB (#ab16502) and histone H3 (#ab8580) were from Abcam (Abcam, UK). Antibodies against eIF2α (#11233-1-AP), GRP78 (#11587-1-AP), TNF-α (#60291-1-Ig), IL-6 (#21865-1-AP) and IL-1β (#26048-1-AP) were from Proteintech Biotechnology (Wuhan, China).

Fatty-acid-supplemented medium was prepared with slight modification of the protocol of Spector (Spector, 1986). Briefly, PA was dissolved in ethanol and then gently mixed until completely dissolved, after which the clear fatty acids solution was complexed with fatty-acid-free BSA at a fatty acid to BSA ratio of 1:10. The complex fatty acid solution was added to the serum-containing cell culture medium to obtain the indicated final PA concentration. The control (untreated) cells received the same vehicle solution but without PA.

2.2. Experimental animals and protocols

All animal experiments were preformed according to the guidelines of Animal Care and Use Committee of Shanghai Jiaotong University. Male Sprague-Dawley rats (weighing 100–110 g), normal chow and the high-fat diet (HFD) chow (rodent regular chow diets supplemented with 20% lard and 3% cholesterol), were all purchased from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). Rats allocated to the HTG group were fed HFD for 2 weeks, whereas rats used as controls were fed with normal chow. AP model was induced by intraperitoneal injection of caerulein, at a dose of 50 μg/kg body weight, two times with an interval of 1 h between injections. The control rats were given the same volume of vehicle solution at the same time point. All rats were sacrificed 9 h after AP induction. Blood samples were collected *via* the abdominal aorta. A portion of each pancreas was fixed in 10% formaldehyde in phosphate-buffered saline (PBS; pH7.4) and embedded in paraffin, and part of the pancreatic tissues were quickly frozen with liquid nitrogen and stored at $-80\,^\circ$C until use.

2.3. Pancreatic acinar cells isolation and treatment

PACs were isolated from mice by a collagenase digestion procedure as described previously (Hu et al., 2011). For treatments, the isolated PACs were incubated at 37 $^\circ$C in Dulbecco's modified Eagle's medium/Ham F-12 medium containing 20% fetal bovine serum and other agents as described below.

In the PA-stimulated group, PACs were incubated with 0.1 mM PA for 1, 3, 6, 9, or 12 h prior to incubation with 20 pM CCK-8 (30 min); vehicle solution was used for the control group. In the 4-PBA group, PACs were pre-incubated with 4-PBA (2.5, 5, 10, or 20 μM) for 30 min and subsequently incubated with 0.1 mM PA or vehicle solution (control) for 6 h prior to incubation with 20 pM CCK-8 (30 min). In the thapsigargin-stimulated group, PACs were incubated with thapsigargin (0.1, 0.3, 1, or 2 μM) for 6 h. In the C/EBPβ small interfering RNA (siRNA) transfection groups, PACs were incubated with 0.1 mM PA for 6 h after transfection.

2.4. Small interfering RNA transfection

For siRNA transfections, primary rat PACs were transfected with 150 nM siRNA oligo-nucleotides using Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's protocol. Transfected cells were tested for their response to PA at 24 h after the initiation of siRNA transfection. The siRNA sequences used for transfection are given in Supplementary Table S1 in the online version at DOI: 10.1016/j.biocel.2016.08.035.