Redox Biology 14 (2018) 295–304

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Impairment of Akt activity by CYP2E1 mediated oxidative stress is involved in chronic ethanol-induced fatty liver

Tao Zeng*, Cui-Li Zhang, Ning Zhao, Min-Jie Guan, Mo Xiao, Rui Yang, Xiu-Lan Zhao, Li-Hua Yu, Zhen-Ping Zhu, Ke-Qin Xie*

Institute of Toxicology, School of Public Health, Shandong University, China

ARTICLE INFO

Keywords:
Alcoholic fatty liver
Protein kinase B
Cytochrome P4502E1
Oxidative stress
Insulin-like growth factor-1

ABSTRACT

Protein kinase B (PKB/Akt) plays important roles in the regulation of lipid homeostasis, and impairment of Akt activity has been demonstrated to be involved in the development of non-alcoholic fatty liver disease (NAFLD). Previous studies suggest that cytochrome P4502E1 (CYP2E1) plays causal roles in the pathogenesis of alcoholic fatty liver (AFL). We hypothesized that Akt activity might be impaired due to CYP2E1-induced oxidative stress in chronic ethanol-induced hepatic steatosis. In this study, we found that chronic ethanol-induced hepatic steatosis was accompanied with reduced phosphorylation of Akt at Thr308 in mice liver. Chronic ethanol exposure had no effects on the protein levels of phosphatidylinositol 3 kinase (PI3K) and phosphatase and tensin homologue deleted on chromosome ten (PTEN), and led to a slight decrease of phosphoinositide-dependent protein kinase 1 (PDK-1) protein level. Ethanol exposure resulted in increased levels of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)-Akt adducts, which was significantly inhibited by chlormethiazole (CMZ), an efficient CYP2E1 inhibitor. Interestingly, N-acetyl-L-cysteine (NAC) significantly attenuated chronic ethanol-induced hepatic fat accumulation and the decline of Akt phosphorylation at Thr308. In the in vitro studies, Akt phosphorylation was suppressed in CYP2E1-expressing HepG2 (CYP2E1-HepG2) cells compared with the negative control HepG2 (NC-HepG2) cells, and 4-HNE treatment led to significant decrease of Akt phosphorylation at Thr308 in wild type HepG2 cells. Lastly, pharmacological activation of Akt by insulin-like growth factor-1 (IGF-1) significantly alleviated chronic ethanol-induced fatty liver in mice. Collectively, these results indicate that CYP2E1-induced oxidative stress may be responsible for ethanol-induced suppression of Akt phosphorylation and pharmacological modulation of Akt in liver may be an effective strategy for the treatment of ethanol-induced fatty liver.

1. Introduction

Alcoholic liver disease (ALD) is a progressively aggravated liver disease, which ranges from steatosis to hepatitis, fibrosis, and finally cirrhosis [1]. Alcoholic fatty liver (AFL) is the earliest and most common phenotype of ALD, and continued drinking of excessive amounts of alcohol can subsequently lead to severe forms of ALD. AFL has been considered as a benign condition for a long time due to the asymptomatic and reversible characteristics. However, increasing evidences suggest that AFL is a potentially pathologic condition [2,3]. AFL could progress to fibrosis and cirrhosis in about 5–15% of AFL patients despite abstinence; and the severity of steatosis on the initial liver biopsy predicted the development of cirrhosis on the subsequent biopsy 10 year later [4,5]. Animal studies demonstrated that fatty liver was more vulnerable to hepatotoxins such as lipopolysaccharide [6]. Now, it is generally recognized that AFL is the optimal phase to block or delay the progress to advanced ALD [7,8].

Protein kinase B (PKB/Akt) is a central player in the signal transduction pathways activated in response to many growth factors, hormones, cytokines, and nutrients [9]. Akt has been described as one of the most important and versatile protein kinases at the core of human physiology and disease [10]. Dysregulated Akt activity is implicated in

Abbreviations: AFL, alcoholic fatty liver; ALD, alcoholic liver disease; ALT, alanine transaminase; AMPK, AMP-activated protein kinase; AST, aspartate transaminase; CMZ, chlormethiazole; CYP2E1, cytochrome P4502E1; 4-HNE, 4-hydroxynonenal; IGF-1, insulin-like growth factor-1; LPS, lipopolysaccharide; MDA, malondialdehyde; NAC, N-acetyl-L-cysteine; NAFLD, non-alcoholic liver disease; PDK-1, phosphoinositide- dependent protein kinase 1; PI3K, phosphatidylinositol 3 kinase; PKB/Akt, protein kinase B; PPAR-α, peroxisome proliferators-activated receptor α; PPAR-γ, peroxisome proliferators- activated receptor γ; PTEN, phosphatase and tensin homologue deleted on chromosome ten; ROS, reactive oxygen species; SIRT-1, sirtuin 1; SREBP-1c, sterol regulatory element-binding protein 1c; TG, triglyceride

* Correspondence to: Institute of Toxicology, School of Public Health, Shandong University, 44 Wenhua West Road, Jinan, Shandong Province, China.
E-mail addresses: zengtao@sdu.edu.cn (T. Zeng), keqinx@sdu.edu.cn (K.-Q. Xie).

http://dx.doi.org/10.1016/j.redox.2017.09.018
Received 7 September 2017; Accepted 24 September 2017
Available online 27 September 2017

the pathogenesis of a growing number of disorders [9]. The roles of Akt on lipid homeostasis have been investigated in several studies. Although results of in vitro studies showed that Akt activation promoted fat accumulation by activating sterol regulatory element binding protein 1c (SREBP-1c) [11,12], in vivo studies revealed that Akt played protective roles against fatty liver. For example, hepatic fat accumulation in rats with high-fat diet-induced nonalcoholic fatty liver disease (NAFLD) was accompanied with reduced phosphorylation of Akt, and pharmacological inhibitor of Akt led to significant fat accumulation in rat liver [13]. Some other studies demonstrated that Akt activation could ameliorate hepatic steatosis in lean mice, ob/ob mice, NAFLD mice, and diabetic mice [14–16]. Results of these studies clearly demonstrate that Akt plays important roles in regulating the lipid homeostasis in the liver. Thus, it is necessary to investigate the roles of Akt in the pathogenesis of AFL.

Microsomal cytochrome P4502E1 (CYP2E1) is a member of the cytochrome P450 mixed-function oxidase system, which is responsible for the metabolism of many endogenous and xenobiotic substrates. Previous studies have demonstrated that CYP2E1 plays etiological roles in the development of AFL [17–19]. However, the underlying mechanisms for CYP2E1 activation and subsequent fat accumulation in the liver are not fully elucidated. It has been well documented that CYP2E1 activation leads to the generation of large amounts of reactive oxygen species (ROS), which can initiate the autocatalytic degradation of polyunsaturated fatty acids to yield electrophilic aldehyde species including 4-hydroxynonenal (4-HNE), which can preferentially modify cysteine, histidine, and lysine residues via Michael addition and for lysine, Schiff base products [20,21]. Indeed, some previous studies have demonstrated that ethanol-induced 4-HNE modification of hepatocellular proteins resulted in the inhibition of normal enzyme function [22,23]. However, the relationship between CYP2E1 and Akt has not been investigated.

The current study was designed to investigate the roles of Akt in the pathogenesis of AFL. We aimed to explore: 1) whether the hepatic Akt activity was impaired in chronic ethanol-exposed mice? 2) if so, whether pharmacological activation of Akt could attenuate chronic ethanol-induced fat accumulation in mice? And 3) the potential links between Akt suppression and ethanol-induced activation of CYP2E1.

2. Materials and methods

2.1. Materials

Ethanol was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Chlormethiazole (CMZ) and N-acetyl-L-cysteine (NAC) were bought from Sigma (St. Louis, MO, USA). Specific primary antibodies against Akt, p-Akt^{ser473}, p-Akt^{thr308}, acyl-CoA carboxylase (ACC), p-ACC^{ser79}, glycogen synthase kinase-3β (GSK-3β), p-GSK-3β^{ser9}, phosphatidylinositol 3 kinase (PI3K)-p110α and PI3K-p85 were bought from Cell Signaling Technology (Beverly, MA, USA). Primary antibodies against CYP2E1, liver fatty acid-binding protein (LFABP), acyl-CoA oxidase (ACOX) and 4-HNE were provided by Abcam (Cambridge, UK). Primary antibodies against SREBP-1c, peroxisome proliferator-activated receptor a (PPAR-a), peroxisome proliferator-activated receptor γ (PPAR-γ), and fatty acid synthase (FAS) were obtained from Santa Cruz (Santa Cruz, CA, USA). Insulin-like growth factor (IGF-1) was obtained from Sino Biological Inc. (Beijing, China). Specific primary antibodies against phosphatase and tensin homologue deleted on chromosome ten (PTEN) and phosphoinositide-dependent protein kinase 1 (PDK-1) were bought from Proteintech (Chicago, IL, USA). 4-HNE was purchased from Calbiochem (San Diego, CA, USA). Malondialdehyde (MDA) and triglyceride (TG) assay kits were supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) and Applygen Technologies Inc. (Beijing, China), respectively. All other reagents were purchased from Sigma unless indicated otherwise.

2.2. Animal treatment

Specific pathogen free (SPF) KM mice (male, 8 weeks old) were provided by Laboratory Animal Center of Shandong University (Jinan, China). The mice were maintained in a temperature-controlled environment (20–22 °C) with a 12-h light: 12-h dark cycle and 50–60% humidity. Chronic AFL was induced by feeding mice with regular Lieber-DeCarli liquid diet containing 5% (w/v) ethanol for 4 weeks. To explore the potential links between ethanol-induced CYP2E1 activation and Akt suppression, chlormethiazole (CMZ, 50 mg/kg body weight), an efficient inhibitor of CYP2E1, was injected intraperitoneally to mice every other day as previously reported [17]. Pharmacological activation of Akt was achieved by intraperitoneal administration of recombinant human insulin-like growth factor-1 (IGF-1, 100 μg/kg body weight) [24]. NAC was administered to mice (100 mg/kg bw) to evaluate the effects of antioxidant on chronic ethanol-induced steatosis. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the U.S. National Institutes of Health and were approved by the Ethics Committee of Shandong University Institute of Preventive Medicine.

2.3. Cell culture and treatment

Human hepatocarcinoma cell line (HepG2) was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). A CYP2E1 cDNA plasmid was kindly provided by Dr. F.J. Gonzalez (National Cancer Institute, Bethesda, MD, USA). A monoclonal cell line stably expressing CYP2E1 (CYP2E1-HepG2) was established by transfecting HepG2 cells with recombinant lentiviral vector (Shanghai Genechem Co., Shanghai, China), while a negative control cell line (NC-HepG2) was also obtained by transfecting HepG2 cells with GFP lentiviral vector. The preparation of the recombinant lentiviral vectors and the transfection procedure were performed as previously reported [25].

The wild type HepG2 cells were grown in DMEM medium (GIBCO BRL, NY, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA) in a humidified incubator set with 5% CO2/95% air atmosphere at 37 °C. The CYP2E1-HepG2 and NC-HepG2 cells were cultured in the same medium supplemented with 2 μg/mL puromycin. HepG2 cells were exposed to 25 μM 4-hydroxynonenal (4-HNE) for a time course from 1 h to 8 h. CYP2E1-HepG2 and NC-HepG2 cells were treated with 0, 25, 50, 100, and 200 mM ethanol for 5 d. To minimize the evaporation of ethanol in the medium, fresh medium containing ethanol was replaced every 24 h. Cytotoxicity of ethanol and 4-HNE was tested using CCK-8 kits (Dojin Laboratories, Kumamoto, Japan). The doses of ethanol and 4-HNE used in this study did not induce significant cytotoxicity.

2.4. Biochemical analyses

The levels of alanine transaminase (ALT), aspartate transaminase (AST) and triglyceride (TG) in serum were measured using GLAMOUR 1600 automatic biochemistry analyzer with commercial assay kits provided by BioSino Biotechnology and Science, Inc (Beijing, China). TG levels in mice liver and cultured cells were determined using TG assay kits obtained from Applygen Technologies Inc. (Beijing, China).

2.5. Determination of hepatic lipid peroxidation

Hepatic lipid peroxidation was evaluated using the thiobarbituric acid reactive substances method (TBARS) and was expressed as malondialdehyde (MDA) levels [26]. To determine the hepatic MDA level, liver tissues were homogenized in 9 volumes of cold buffer (10 mM Tris, 100 μM EDTA, 10 mM saccharose, 0.8% saline, pH 7.4). The homogenates were centrifuged at 1000 × *g* for 15 min at 4 °C, and the supernatant were collected for the detection of MDA using a commercial assay kit provided by Nanjing Jiancheng Bioengineering Institute