Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 55 (2018) 12–25

Journal of Nutritional Biochemistry

Preventive effects of indole-3-carbinol against alcohol-induced liver injury in mice via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms: Role of gut-liver-adipose tissue axis

Youngshim Choi*, Mohamed A. Abdelmegeed, Byoung-Joon Song*

Section of Molecular Pharmacology and Toxicology, Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA

Received 14 July 2017; received in revised form 1 October 2017; accepted 14 November 2017

Abstract

Indole-3-carbinol (I3C), found in *Brassica* family vegetables, exhibits antioxidant, anti-inflammatory, and anti-cancerous properties. Here, we aimed to evaluate the preventive effects of I3C against ethanol (EtOH)-induced liver injury and study the protective mechanism(s) by using the well-established chronic-plus-binge alcohol exposure model. The preventive effects of I3C were evaluated by conducting various histological, biochemical, and real-time PCR analyses in mouse liver, adipose tissue, and colon, since functional alterations of adipose tissue and intestine can also participate in promoting EtOH-induced liver damage. Daily treatment with I3C alleviated EtOH-induced liver injury and hepatocyte apoptosis, but not steatosis, by attenuating elevated oxidative stress, as evidenced by the decreased levels of hepatic lipid peroxidation, hydrogen peroxide, CYP2E1, NADPH-oxidase, and protein acetylation with maintenance of mitochondrial complex I, II, and III protein levels and activities. I3C also restored the hepatic antioxidant capacity by preventing EtOH-induced suppression of glutathione contents and mitochondrial aldehyde dehydrogenase-2 activity. I3C preventive effects were also achieved by attenuating the increased levels of hepatic proinflammatory cytokines, including IL1β, and neutrophil infiltration. I3C also attenuated EtOH-induced gut leakiness with decreased serum endotoxin levels through preventing EtOH-induced oxidative stress, apoptosis of enterocytes, and alteration of tight junction protein claudin-1. Furthermore, I3C alleviated adipose tissue inflammation and decreased free fatty acid release. Collectively, I3C prevented EtOH-induced liver injury via attenuating the damaging effect of ethanol on the gut-liver-adipose tissue axis. Therefore, I3C may also have a high potential for translational research in treating or preventing other types of hepatic injury associated with oxidative stress and inflammation.

Keywords: Indole-3-carbinol; Alcohol; Liver; Inflammation; Oxidative stress; Apoptosis

1. Introduction

Alcoholic beverages are widely consumed all over the world. While moderate alcohol [ethanol (EtOH)] drinking is beneficial for mood enhancing and preventing cardiovascular and neurological diseases [1], chronic excessive alcohol consumption can cause various sociomedical and public health problems worldwide [2,3]. The onset and progression of alcoholic liver disease (ALD) is multifactorial. In the liver, chronic alcohol drinking promotes hepatic steatosis (fat accumulation) and this can progress to more severe forms of liver disease, including liver inflammation [i.e., alcoholic steatohepatitis (ASH)], with a relatively high mortality rate, fibrosis, cirrhosis, hepatocarcinoma and hepatic failure [2,4]. These facts indicate the critical value of preventing the development of ALD at the early stages better than struggling with its treatment at later time points due to relatively short life expectancy of the patients after clinical diagnosis of ASH [2,3]. Hepatocyte ballooning and apoptotic hepatocytes are the common features in the liver of patients with ASH [5]. Excessive hepatocyte apoptosis stimulates inflammation and stimulates infiltration of phagocytic neutrophils and/or the production of proinflammatory cytokines and reactive oxygen species (ROS) by innate immune cells [6]. Additionally, oxidative stress-induced hepatocyte apoptosis represents one of the consequences of acute alcohol injury [7]. Thus, it seems like that there is a vicious cycle of hepatocyte apoptosis and immune cell activation that promotes the progression of liver injury in ALD.

Direct injurious effects by ethanol on the liver via increasing hepatic oxidative stress, inflammation, and cell apoptosis might not be the only route via which ethanol produces its deleterious consequences. Recently emerging reports show that adipose tissue dysfunction can influence hepatic metabolism [8]. Ethanol is also known to stimulate adipose tissue inflammation, apoptosis of adipocytes, and release of free fatty acids (FAAs) [9], all of which can participate in EtOH-induced liver injury. Additionally, It is now widely-accepted that endotoxin, released from the gut into portal

* Corresponding authors at: Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcohol Abuse and Alcoholism, 9000 Rockville Pike, Bethesda, MD 20892-9410, USA. Tel.: +1 301 496 3985; fax: +1 301 594 3113.

E-mail addresses: youngshimchoi@gmail.com (Y. Choi), bj.song@nih.gov (B.-J. Song).

https://doi.org/10.1016/j.jnutbio.2017.11.011
0955-2863/

circulation due to compromised intestinal integrity, plays an important role in mediating inflammatory reactions and advancing liver injury, including cell apoptosis, in response to ethanol [10–14]. Thus, both adipose tissue and gut are likely to play an important role in the development and/or the progression of ALD.

Recently, the beneficial effects of natural compounds with antioxidant capacities on preventing ALD in experimental models have been reported, and the bioactive, protective compounds predominantly belong to polyphenols, including flavonoid compounds [15–19]. Indole-3-carbinol (I3C) is a naturally occurring compound produced from glucobrassicin in Brassica (cruciferous) vegetables such as cabbage, broccoli, cauliflower and brussel sprouts. As a nutritional supplement, I3C has received much attention lately due to its promising preventive and treatment properties against various types of cancer [20].

I3C has been suggested as a potential anti-obesity and anti-inflammatory agent [21]. For instance, I3C improved hyperglycemia and hyperinsulinemia in mice fed a n-6 fatty acid containing high-fat diet (HFD) and decreased the expression of many proinflammatory cytokines. The protective action of I3C against HFD-induced hepatic steatosis is mediated, at least in part, through the up-regulation of the sirtuin1 (SIRT1)-AMP-activated protein kinase (AMPK) signaling pathway in the livers of HFD-fed mice [22]. However, the preventive benefits of I3C against the development of ALD have been poorly characterized.

An ongoing challenge for the management of ALD, including ASH with a relatively high rate of mortality [2,3,23], is the understanding of the early liver injury mechanisms and the identification of a dietary supplement that can prevent this process at early stages. This is particularly important since early intervention strategies can prevent serious and irreversible damage and deaths. The real dilemma is that many alcoholics cannot control their drinking habits due to addiction and/or physical dependency and still suffer from various medical conditions including ALD with inflammation. Consequently, identification of a safe and preventive agent against ALD is still needed. The aims of this study were: (1) To examine the hypothesis that I3C can prevent or minimize the development of ALD via anti-inflammatory and antioxidant mechanisms, (2) to evaluate the hypothesis that I3C preventive effect may extend beyond its direct action on the liver by evaluating other organs such as adipose tissue and intestine, which are known to contribute to the liver damage, and (3) to establish the pathological role of the gut-liver-adipose tissue axis in promoting ALD. To achieve these aims, we used a well-established model, namely chronic-plus-single-binge ethanol exposure model [24] in the absence or presence of the I3C treatment. Our findings show that I3C may represent a novel, protective strategy against alcoholic liver injury by attenuating oxidative stress, inflammatory response, and apoptosis and that this preventive action is mediated, at least partially, through the gut-liver-adipose tissue axis.

2. Materials and methods

2.1. Materials

All chemicals used in this study were purchased from Sigma Chemical (St. Louis, MO, USA), unless indicated otherwise. Specific antibodies against CYP2E1 (catalog #ab28146), inducible nitric oxide synthase (iNOS) (catalog #ab3523), 3-nitrotyrosine (3-NT) (catalog #ab7084), nicotinamide adenine dinucleotide phosphate oxidase-4 (NADPH-oxidase) (catalog #ab133303), total OXPHOS (catalog #ab110413), IL1β (catalog #9722), osteopontin (OPN) (catalog #ab8448, Ly6g (catalog #ab25377), and claudin-1 (catalog #ab15098) were from Abcam (Cambridge, MA, USA). Specific antibodies against poly(ADP-ribose) polymerase-1 (PARP-1) (catalog #sc7150), mitochondrial aldehyde dehydrogenase-2 (ALDH2) (catalog #sc48838), ATP synthase subunit beta (ATP5B) (catalog #sc16690), and secondary antibodies conjugated with horse radish peroxidase were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against acetylated lysine (catalog #9441S) and β-actin antibody (catalog #4967) were from Cell Signaling Technology, Danvers, MA, USA, while anti-myeloperoxidase antibody (MPO) (catalog #SKU: PP023AA) was purchased from Biocare Medical, Concord, CA, USA.

2.2. Animals and experimental design

Age-matched (8–10 weeks of age) male C57BL/6 J mice were obtained from Jackson Laboratories. After one week of acclimation, the mice were randomly divided into four groups (n=10/group): (1) Control, (2) control + I3C, (3) EtOH, and (4) EtOH + I3C. The mice were fed the Lieber-DeCarli control (dextrose) liquid diet *ad libitum* for 5 days to allow mice to acclimate to the liquid diet and tube feeding. Mice were then subjected to the chronic-plus-single binge ethanol exposure model [24]. Lieber-DeCarli '82 Shake and Pour control liquid diet (Bio-Serv, product no. F1259SP) and Lieber-DeCarli '82 Shake and Pour ethanol liquid diet (Bio-Serv, product no. F1258SP) were used for diet preparation. Both the control + I3C and EtOH + I3C groups were subjected to I3C (40 mg/kg body weight/day) via daily oral gavage during the acclimation period and throughout the liquid diet feeding period. Afterward, alcohol-fed mice were allowed free access to the Lieber-DeCarli alcohol liquid diet containing 5% (vol/vol) ethanol for 10 days, and control groups were pair-fed with the isocaloric dextrose-control liquid diet, as described [24]. To ensure the adequate I3C delivery, mice were treated with freshly-prepared I3C once a day by oral gavage at a dose of 40 mg/kg body weight/day just before the daily administration of the ethanol or dextrose-control liquid diet. This I3C oral dosage was well tolerated and exhibited preventive effects on hyperglycemia-induced oxidative stress in mice treated for 35 days [25]. In the early morning on day 11, both alcohol-fed and pair-fed control mice were exposed to a single oral (gavage) dose of alcohol (5 g/kg BW) or isocaloric dextrose, respectively, and euthanized 9 h later for tissue collection. Both groups were subjected to a final dose of I3C (40 mg/kg body weight) 1.5 h prior to the last oral dose of binge ethanol (5 g/kg) or dextrose. Liver, epididymal adipose tissues, colon, and serum were collected from each mouse. Parts of the tissues (liver, epididymal adipose tissue, and colon) were fixed in 10% formalin for histological analysis, while the rest of the tissues and the serum were quickly frozen and stored at −80 °C until analysis. Animal experiments were performed in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee.

2.3. Immunoblots analysis for detecting target proteins

Total liver homogenates were prepared in ice-cold extraction buffer (50 mM Tris–Cl, pH 7.5, 1 mM EDTA, and 1% CHAPS), while mitochondrial fractions were prepared by differential centrifugation, as described previously [26–28]. The extraction buffers were subjected to nitrogen gas to remove the dissolved oxygen. The bicinchoninic acid protein assay reagent (Pierce, Rockford, IL, USA) was used to measure protein concentration for each sample (total protein lysate or mitochondrial protein). Equal amounts of lysates were subjected to 10% SDS-PAGE gels and transferred to nitrocellulose membranes (Hercules, CA, USA). Membranes were blocked with 5% (w/v) nonfat milk proteins. Following the washing steps, membrane was exposed to a primary antibody specific for the target protein in 3.5% (w/v) bovine serum albumin (BSA) overnight at 4°C. Membranes were then washed three separate times to remove the primary antibody and subjected to incubation with the secondary antibody IgG conjugated with horse radish peroxidase (Santa Cruz Biotechnology, Dallas, TX, USA) for 1 h at room temperature. Image was detected by SuperSignal West Pico Kit (Thermo Fisher Scientific, Waltham, MA). β-Actin was used as a loading control for whole liver extracts, while ATP5B was used for mitochondrial proteins. Intestinal homogenates were prepared in RIPA buffer (Thermo Fisher Scientific, Waltham, MA), followed by sonication. Lysates were then subjected to centrifugation 10,000 x *g* for 20 min and the supernatant was used for analysis, and β-actin was used as a loading control.

2.4. Biochemical assays

Liver tissues (50 mg wet weight) were homogenized in 5% Triton X-100 solution and heated in 80–100 °C water bath for 2–5 min to solubilize the triglyceride (TG). The samples were then centrifuged at 10,000×*g* for 10 min, and the resulting supernatant was used to determine the TG level by following the manufacturer's protocol (catalog #10010303), (Cayman Chemical, Ann Arbor, MI, USA). Serum concentrations of alanine aminotransferase (ALT) (catalog #700260) (Cayman Chemical, Ann Arbor, MI, USA). Serum concentrations of free fatty acid (FFA) (catalog #K612-100) were determined enzymatically using commercial kits (BioVision, Milpitas, CA, USA). Commercial kits (catalog #MAKO55-1KT, Sigma Aldrich, MO, USA) were used to measure the levels of serum aspartate aminotransferase (AST) and serum ethanol (catalog #ab65343, Abcam, Cambridge, MA, USA), respectively. Serum adiponectin and leptin concentrations were measured using the ELISA kits (catalog #KMP0041 and catalog #KMP0041, respectively) (Thermo Fisher Scientific, Waltham, MA, USA). Serum endotoxin levels were measured using the commercial kit from Lonza (catalog #50-647U) (Walkersville, MD, USA). Commercial kits were used to measure caspase-1 (catalog #K111–100), hydrogen peroxide (H_2O_2) (catalog #K265) (BioVision, Milpitas, CA, USA), caspase-3 (catalog #ab39401) (Abcam, Cambridge, MA, USA), and glutathione (GSH) levels (catalog #703002) (Cayman chemical, Ann Arbor, MI, USA) in liver homogenate by following the manufacturer's protocols. Commercially available kits were used to determine the activities of mitochondrial complexes I (catalog #700930), II and III (catalog #700950) (Cayman Chemical, Ann Arbor, MI, USA), and mitochondrial ALDH2 (catalog