European Journal of Pharmacology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Fructose 1, 6-diphosphate prevents alcohol-induced liver injury through inhibiting oxidative stress and promoting alcohol metabolism in mice

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ARTICLE INFO

Keywords:
Fructose 1, 6-diphosphate
Alcoholic liver disease
Antioxidant capability
Cell apoptosis

ABSTRACT

Fructose 1, 6-diphosphate (FDP), a glycolytic intermediate , has been identified to possess antioxidant activities. Here we show the protective effect of FDP against alcohol-induced liver injury in mice and the underlying mechanisms. The in vivo experiments demonstrated that FDP, orally administered to mice, dose-dependently suppressed alcohol (50%, v/v, 12 ml/kg)-induced increase of serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serum triglyceride (TG) level and hepatic malondialdehyde (MDA) level. FDP also inhibited liver histological lesions induced by seven-day administration of alcohol to mice. *In vitro* study indicated that FDP inhibited ethanol-induced L02 cell apoptosis via reducing pro-caspase3 protein level and increasing poly ADP-ribose polymerase (PARP) cleavage. The mechanism analysis showed that FDP prevented ethanol-induced decrease of mouse antioxidant capability through inhibiting the reducion of the level of glutathione (GSH) and activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GSH-PX) in mouse livers, and suppressing the reducion of GSH level and SOD activity in L02 cells. FDP also enhanced alcohol metabolic rate through increasing alcohol dehydrogenase (ADH) activity and acetaldehyde dehydrogenase (ALDH) protein level, and down-regulating cytochrome p450 2E1 (CYP2E1). These results displayed that FDP protected mice from alcohol-induced liver injury, suggesting the potential activity of FDP in preventing alcoholic liver disease (ALD).

1. Introduction

ALD is induced by the chronic and excessive consumption of alcoholic beverages, which has been considered to comprise three main stages, including steatosis, alcohol hepatitis and chronic hepatitis with progressive fibrosis or cirrhosis, and these stages may sometimes coexist in one individual (Masarone et al., 2016). Among all liver diseases, alcoholic liver cirrhosis and other ALDs represent the larger part, but it is difficult to exactly know the global burden of ALD (Masarone et al., 2016). According to the demonstration in 2010 Global Burden of Diseases Reports, in the worldwide, about 47.9% of all liver cirrhosis deaths were attributable to alcohol, and mortality from alcoholic liver cirrhosis was of 7.2 per 100.000 inhabitants (Lozano et al., 2013). In China, due to increased frequency of drinking and the increase of dietary fat intake, the incidence of ALD has increased, becoming another important risk factor in addition to viral hepatitis (Ramirez et al., 2017).

Alcohol is primarily metabolized in the liver through three enzymatic pathways: ADH, ALDH and CYP2E1. Excessive alcohol consumption leads to the accumulation of the highly toxic acetaldehyde which may damage membrane lipids and alter enzyme activities (Salaspuro et al., 1981). Although the progression of liver injury induced by alcohol is a multifactorial event that involves genetic and environmental factors, the oxidative stress has been reported to play an essential role in ALD (Kono et al., 2000). The alcohol exposure may damage liver enzymatic and non-enzymatic mechanisms which may protect cells from toxicity of reactive oxygen species (Wu and Cederbaum, 2003). Accumulating evidence has proposed that mitochondrial injury connected with cell apoptosis is responsible for the progression of ALD (Stewart et al., 2001; Yan et al., 2007), and both release of cytochrome c and caspase activation has been demonstrated to be involved in mitochondrial apoptotic pathway (Susin et al., 1998).

FDP, a glycolytic intermediate, has been reported to attenuate tissue injury associated with ischemia and shock via promoting the anaerobic

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http://dx.doi.org/10.1016/j.ejphar.2017.09.034
Received 15 August 2017; Received in revised form 19 September 2017; Accepted 20 September 2017

carbohydrate utilization and decreasing oxygen-free-radical generation in neutrophils (Didlake et al., 1989; Farias et al., 1990; Karaça et al., 2002). FDP also can activate the pentose phosphate pathway (PPP) for anaerobic ATP production (Kelleher et al., 1995). Since FDP was found to maintain cellular levels of glutathione and catalase in addition to upregulating the activity of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of PPP, it is likely that FDP suppresses oxidative stress through PPP activation (Ahn et al., 2002). Recently, it has been reported that FDP can decrease oxidative stress by limiting free radical production and improving antioxidant systems (Alva et al., 2016).

Here we showed protective effect of FDP on mice against alcohol-induced acute liver injury and probed the possible mechanism.

2. Materials and methods

2.1. Chemicals, assay kits and antibodies

Commercial assay kits for activities of ALT, AST, SOD, GSH-PX, CAT, GR and ADH, and assay kits for MDA, GSH and TG level detection were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Ethyl alcohol was from Guangdong Guanghua Sci-Tech Co., Ltd. (Guangdong, China). Polyclonal antibody against procaspase3 was from Abcam (Cambridge, UK). PARP antibody was purchased from Cell Signaling Technology (Beverly, USA). Monoclonal antibody to β-actin and all secondary antibodies were purchased from Bioworld Biotechnology (Jiangsu, China). FDP was purchased from Shanghai Hanhong Scientific Co., Ltd (Shanghai, China).

2.2. Animals and experimental design

Male ICR mice (6–7 weeks, 18–22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. And all mice received human care in strict accordance with the requirements of Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation and were approved by Science and Technology Department of Jiangsu Province.

After 2–3 days acclimatization to standard laboratory conditions, mice were randomly divided into five groups (eight mice/group) as follows. Mice in Group I (served as normal control) were orally administered with sterile distilled water only during the experimental period. Group II was the model group, in which mice were given sterile distilled water orally and followed by administration of ethanol (50%, v/v, 12 ml/kg) by gavage after 1 h. Mice in groups III, IV and V were orally administered with FDP at 62.5, 125 and 250 mg/kg respectively and then received alcohol (50%, v/v, 12 ml/kg) challenge. The whole blood of mice was collected by retroorbital bleeding 6 h after ethanol administration and sera were obtained by centrifugation (1750 g, 15 min, 4 ℃) for measuring the activities of ALT and AST, and level of TG. The livers were removed quickly for detecting hepatic level of MDA and GSH as well as activities of antioxidant enzymes.

To observe the effect of FDP on alcohol-induced histological changes, mice were administrated with ethanol (50%, v/v, 12 ml/kg/day) for seven days and FDP was given to mice 1 h before alcohol treatment. During this process the mouse body weight was monitored , and at end of the treatment , the weight of organs were measured and the livers were collected for histological observation. Meanwhile, the content of alcohol in mouse blood was detected by gas chromatography 30, 90 and 180 min after the last administration of ethanol.

2.3. Measurement of serum activities of ALT and AST, and level of TG

Activities of ALT and AST and the level of TG in mouse sera were detected spectrophotometrically using the commercial assay kits in accordance with the manufacture's protocols. The absorbance values were determined at corresponding wavelength with an Elx 800 Universal Microplate Reader (BIO-TEK, INC).

2.4. Determination of the activities of antioxidant and alcohol metabolic enzymes, and lipid peroxidation

Liver tissues were homogenized in cold 0.9% NaCl solution and were centrifuged (1000 g, 15 min, 4 ℃). The supernatants were prepared for the further detection. The activities of antioxidant enzymes including SOD, CAT, GR, GSH-PX and ADH, and the level of GSH were determined spectrophotometrically by using the commercial assay kits according to the instructions supplied by manufacturer. Lipid peroxidation in liver was determined spectrophotometrically through measuring thiobarbituric acid-reactive substances (TBARS) using the detection kits in accordance with the manufacturer's protocols, and expressed as malondialdehyde (MDA) concentration. The level of ALDH in mouse liver homogenate was determined according to the standard procedures of the commercial ELISA kits (Threebio Technology, China).

2.5. Cell culture and viability assay

The human hepatic cell strain, L02 obtained from Key GEN Bio TECH Corp., Ltd (Jiangsu, China), was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (100U/ml penicillin and 100 μg/ml streptomycin), in a 5% CO_2 humidified environment at 37 ℃. For cell viability assay , L02 cells were seeded into 96-well plates at a density of 5×10^3 cells / well 24 h before treatment. The cell viability was determined using the MTT [3-(4, 5-dimethylthia-zol-2-yl) − 2, 5-diphenyltetrazolium bromide] commercial assay kit. Briefly, 10 μl (5 mg/ml) MTT working solution was added to each well, and after 4 h, the supernatant was removed and 200 μl of dimethylsulfoxide (DMSO) was added to dissolve the crystals. Absorbance of each well was detected at 570 nm using an Elx 808 Universal Microplate Reader (BIO-TEK, INC).

2.6. Flow cytometric analysis

The apoptosis of L02 cells was evaluated through using the Annexin V-FITC/PI apoptosis detection kit (Vazyme Biotech, China). Briefly, cells were collected by centrifuge and washed with PBS, and then were resuspended in 100 μl binding buffer followed by incubation with 5 μl Annexin V-FITC and 5 μl PI for 15 min at room temperature in the dark. After adding 400 μl binding buffer, the apoptotic cells were assessed using a flow cytometer (BD Biosciences, USA).

2.7. Western blot analyses

Cells were washed with cold PBS and lysed in the lysis buffer for 30 min and then were centrifugated (15,000 g, 4 ℃, 15 min). Equal amounts of the soluble protein were denatured by treatment with sodium dodecyl sulfate (SDS), electrophoresed on 12% SDS-polyacrylamide gradient gels, and transferred to polyvinylidene difluoride (PVDF) membranes. After being washed with Tris-buffered saline (TBS) for 5 min and blocked with 5% skim milk in TBS for 1 h, the membrane was incubated with matched primary antibodies for 18 h at 4 ℃ followed by incubating with the secondary antibodies. The antibody–antigen complexes were visualized by the LI-COR Odyssey Infrared Imaging System and the blot quantification was performed by using the LI-COR Odyssey analysis software.

2.8. Histological and immunohistochemical analysis

The mouse livers were fixed with 4% paraformaldehyde followed by embedding in paraffin. Sections of 3 μm thickness were cut and stained with hematoxylin-eosin (H & E) or oil red O according the regular staining protocol. The results were evaluated by observing every section under a microscope (OLYMPUS CKX41, Japan). The ethanol induced hepatic injury was graded according to the previous report (Zhao et al., 2017). Grade 0 (no pathological change), grade 1 (minimal hepatocyte