Archives of Biochemistry and Biophysics 629 (2017) 1–7

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Polydatin protects against carbon tetrachloride-induced liver fibrosis in mice

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ARTICLE INFO

Article history:
Received 24 April 2017
Received in revised form
22 June 2017
Accepted 25 June 2017
Available online 27 June 2017

Keywords:
Polydatin
Liver fibrosis
Oxidative stress
Inflammation

ABSTRACT

Reactive oxygen species (ROS) play a key role in chronic liver injury and fibrosis. Polydatin, a glucoside of resveratrol, has been shown to possess anti-oxidative bioactivity. It has been demonstrated that resveratrol has many therapeutic effects on liver disorders including liver fibrosis. Recent study showed that polydatin prevented acute liver injury after carbon tetrachloride (CCl_4) induction. However, the protective effects of polydatin on chronic liver injury and fibrosis has not been understood. Thus, we aimed to determine the roles of polydatin in chronic liver injury and fibrosis. Mice were induced by CCl_4 for 6 weeks to develop chronic liver injury and fibrosis. Mice were treated with polydatin for 3 and 6 weeks, respectively. After 6 week injection of CCl_4, the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were markedly increased. The hepatic expression of α-SMA, collagen deposition and macrophage filtration were also increased. Furthermore, hepatic 4-HNE production and NOX4 expression were also increased in CCl_4-induced mice. In contrast, polydatin ameliorated impaired liver function and histology. Moreover, polydatin attenuated liver fibrosis and inflammation in mice induced by CCl_4. Additionally, polydatin suppressed hepatic 4-HNE production and NOX4 expression. In conclusion, polydatin ameliorate chronic liver injury and fibrosis through inhibition of oxidative stress and inflammation.

1. Introduction

Hepatic fibrosis is the pathological process of liver structure and function under the action of one or more kinds of damage factors, and it is also the necessary stage for the development of various chronic liver diseases to cirrhosis [1]. Upon the activation of hepatic stellate cells (HSCs) by various stimuli, such as transforming growth factor (TGF)-β, quiescent HSCs transdifferentiate into myofibroblasts and then play a key role in the pathogenesis of hepatic fibrosis by producing extracellular matrix proteins, such as type I collagen [2]. Kupffer cells, hepatic resident macrophage, induce acute and chronic liver inflammation by producing inflammatory cytokines including tumor necrosis factor α (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and TGF-β, which further activate HSCs during hepatic fibrosis [3–5]. Numerous studies have demonstrated that the advanced liver fibrosis in patients and in experimental rodent models is reversible [6–8]. However, the current therapies to treat hepatic fibrosis is only to remove the causative agent, it is necessary to develop new specific therapies for liver fibrosis.

Oxidative stress results from an inappropriate balance between the production and clearance of reactive oxygen species (ROS) and leads to aberrant tissue repair in liver. ROS function as key secondary messengers in numerous signaling pathways, contributing to hepatic fibrosis caused by various injuries [9]. Nicotinamide

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http://dx.doi.org/10.1016/j.abb.2017.06.017
0003-9861/

adenine dinucleotide phosphate (NADPH) oxidative (NOX) is an enzyme system that catalyzes the reduction of molecular oxygen to superoxide. Among the NOX family, NOX1 and NOX4 are expressed on HSCs and contribute to liver fibrosis [10,11]. GKT137831, a NOX1/4 inhibitor, attenuated liver fibrosis and ROS production [11]. Treatment targeting NOX may be a new therapy for liver fibrosis.

Polydatin, a resveratrol glucoside (resveratrol-3-O-β-mono-D-glucoside) (Fig. 1), is an active component isolated from the roots of Polygonum with a cuspidatum Sieb. et Zucc [12]. Resveratrol can play a pivotal role in the prevention and treatment of liver fibrosis. Oxidative stress in liver injury is a major pathogenetic factor in progress of liver fibrosis [13]. Previous studies confirmed its anti-oxidative properties in different models of hepatitis resulting in reducing of hepatic fibrosis [13,14]. Polydatin is more resistant to enzymatic oxidation than resveratrol and is soluble in water. Unlike resveratrol, which passively penetrates cells, polydatin enters cells via an active mechanism using glucose carrier [15]. These properties endow polydatin with a greater bioavailability than resveratrol. It has been demonstrated that polydatin had many effects on oxidation and inflammation by attenuation of ROS generation and inflammatory damage [16,17]. Recent report showed that polydatin protected against CCl_4-induced liver injury in mice [18]. However, whether polydatin attenuates liver fibrosis has not been elucidated yet. In the current study, we aimed to assess the effects of polydatin on the liver fibrosis in mice. We hypothesized that polydatin attenuates liver fibrosis through inhibition of oxidative stress and inflammation in liver.

2. Materials and methods

2.1. Materials and reagents

Polydatin was purchased from Sigma (Shanghai, China) and dimethyl sulfoxide (DMSO) was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Carbon tetrachloride and corn oil were obtained from Aladdin (Shanghai, China). Antibodies were obtained from the following sources: Anti-NOX4, anti-α-SMA and anti-CD68 antibodies was from BOSTER (Wuhan, China); horseradishperoxidase (HRP)-conjugated Affinity Pure goat anti-mouse IgG and anti-rabbit IgG were purchased from Zhongshan Golden Bridge Biotechnology Co, Ltd. (Beijing, China). Enhanced chemiluminescence (ECL) substrate for detection of HRP and Protease Inhibitor Cocktail Kit were obtained from Pierce Thermo Scientific (Rockford, USA).

2.2. Animals and treatment

All animal procedures were conducted in accordance with the China Animal Welfare Legislation and were approved by the Ethics Committee on the Care and Use of Laboratory Animals in Guangdong Pharmaceutical University (Guangzhou, China). C57BL/6 mice (20 male and 20 female, 8-week-old, 22–25 g) were purchased from Experimental Animals Center of Guangdong Province, China. They were housed in a temperature-controlled environment (22 ± 2 °C) under standard 12 h light/dark conditions and received food and water ad libitum. Liver fibrosis was induced by intraperitoneally (i.p) injectioin with CCl_4 (diluted at 1:4 in corn oil) (5 ml/kg body weight) twice a week for 6 weeks. Control mice were intraperitoneally injected with corn oil alone. All the treatment groups were injected intraperitoneally with PD (dissolved in saline) at the dose of 5 mg/kg since the 1st and 4th week, respectively (n = 10).

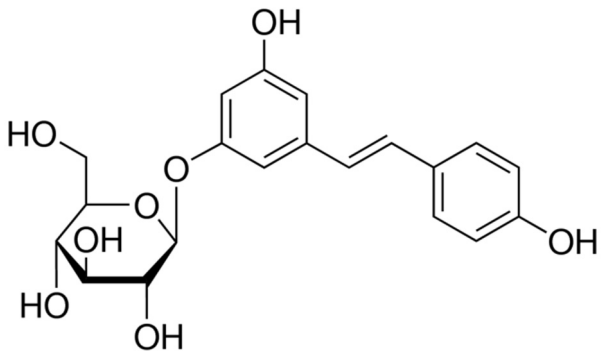

Fig. 1. Molecular structure of polydatin.

2.3. Serum biochemistry

Serum levels of ALT and AST were measured using standard enzymatic procedures according to the manufactures' instruction (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Hydroxyproline assay

Hepatic hydroxyproline content was measured by the methods of alkali hydrolyzation (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Briefly, liver samples (30–100 mg) were hydrolyzed at 100 °C for 20 min, then adjusted pH to 6.0–6.8, then filtered through activated charcoal and centrifuged, adding detecting liquid in 60 °C water bath for 15 min. Samples were measured at 550 nm, and the concentration of total hepatic hydroxyproline was calculated against formula.

2.5. Immunohistochemistry and immunofluorescent staining

Liver specimens fixed in 10% buffered formalin were embedded in paraffin blocks. Liver sections (4 μm thick) were processed using a standard immunostaining protocol. For immunohistochemical analysis, mouse liver sections were separated, rehydrated and incubated with anti-α-SMA and anti-CD68 antibody (1:200, BOSTER, Wuhan). The area of positive staining was measured in high-power (x 20) fields on each slide and quantified using Image J software.

For immunofluorescent staining, the sections were incubated with anti-NOX4 (1:200, BOSTER, Wuhan), anti-4-HNE (1:200, Abcam) antibodies and Alexa Fluor 594-conjugated secondary antibodies (1:200, Zhongshan Golden Bridge Biotechnology) and captured by fluorescent microscopy.

2.6. Liver oxidative stress in mice

Superoxide generation was assessed by quantifying the conversion of dihydroethidium to ethidium. Images were obtained with a Leica TCS SL confocal microscope. Fluorescence was detected with a 585-nm long-pass filter. Laser settings were identical for acquisition of images from all specimens.

2.7. Quantitative real-time RT-PCR

Total RNA was extracted from mouse liver tissues using TRIzol reagent (Takara bio) followed by treatment with RNase-free DNase (Takara, Dalian, China) for 30 min at 37 °C. RNA was reverse-transcribed using a first-strand cDNA kit with random primers (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Real-time quantitative PCR of samples was performed for 40 cycles of 15 s at 95 °C and 60 s at 60 °C using ABI