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Apigenin protects against alcohol-induced liver injury in mice by regulating hepatic CYP2E1-mediated oxidative stress and PPARα-mediated lipogenic gene expression

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ABSTRACT

Alcohol is a major cause of liver injury, and there are currently no ideal pharmacological reagents that can prevent or reverse this disease. Apigenin is one of the most common flavonoids present in numerous plants and has many beneficial effects. But whether or not apigenin may protect against alcohol-induced liver injury remains unknown. Our aim was to examine the effect and potential mechanisms. The experimental mice were given 56% erguotou wine or simultaneously given apigenin 150–300 mg/kg by gavage for 30 days. The results showed that in the apigenin-treated mice, the expression of hepatic cytochrome P450 2E1 (CYP2E1) and nuclear factor kappa B proteins as well as contents of hepatic malondialdehyde and tumor necrosis factor-alpha were reduced, while the levels of hepatic reduced glutathione, glutathione reductase, glutathione peroxidase, and glutathione S-transferase were increased, especially in the 300 mg/kg group. A significant change in hepatic steatosis was also observed in the apigenin 300 mg/kg group. Apigenin pretreatment could increase the expression of hepatic peroxisome proliferator-activated receptor alpha (PPARα) and carnitine palmitoyltransferase-1 proteins, and decrease the expression of hepatic sterol regulatory element binding protein-1c, fatty acid synthase, and diacylglycerol acyltransferase proteins. These findings demonstrated that apigenin might exert a protective effect on alcohol-induced liver injury, and its mechanisms might be related to the regulations of hepatic CYP2E1-mediated oxidative stress and PPARα-mediated lipogenic gene expression.

1. Introduction

Alcohol is a major cause of liver injury, which may extend from simple fatty liver through hepatosteatitis into fibrosis and cirrhosis. Although the mechanisms of alcohol-induced hepatotoxicity are very complex and have not been fully clarified, the hepatic oxidative stress and abnormal lipid metabolism may play the important roles in the process of generation and development [1,2]. Alcohol intake may induce the activation of hepatic cytochrome P450 2E1 (CYP2E1) [3], which subsequently results in the production of reactive oxygen species (ROS) and causes the generation of oxidative stress [4,5]. Under normal physiological conditions, the hepatic antioxidant system can prevent the oxidative stress, but heavy alcoholic exposure may impair the system and lead to the lipid peroxidation [6]. On the other hand, the oxidative stress may also trigger the activation of nuclear factor kappa B (NF-κB) inflammatory pathway in liver [7], which may increase the synthesis and release of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) [8]. Therefore, CYP2E1-mediated oxidative stress

Abbreviations: CPT-1, carnitine palmitoyltransferase-1; CYP2E1, cytochrome P450 2E1; DGAT, diacylglycerol acyltransferase; FAS, fatty acid synthase; GR, glutathione reductase; GSH, reduced glutathione; GSH-PX, glutathione peroxidase; GST, glutathione S-transferase; MDA, malondialdehyde; NF-κB, nuclear factor kappa B; PPARα, peroxisome proliferator-activated receptor alpha; ROS, reactive oxygen species; SREBP-1c, sterol regulatory element binding protein-1c; TNF-α, tumor necrosis factor-alpha.

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plays a key role in the process of alcoholic liver injury [9].

Long-term alcohol consumption can also increase the hepatic lipogenesis and result in the accumulation of fat droplets in hepatocytes [10,11], which may render the liver more susceptible to the oxidative stress [12]. Peroxisome proliferator-activated receptor alpha (PPARα), one of the PPAR receptors, is highly expressed in the liver and involved in the hepatic lipid homeostasis by controlling its target genes including diacylglycerol acyltransferase (DGAT), sterol regulatory element binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and carnitine palmitoyltransferase-1 (CPT-1) [13,14]. So, it is now accepted that the activation of PPARα may improve the hepatic abnormal lipid metabolism induced by alcohol [13,15].

At present, there are no ideal pharmacological reagents that can prevent or reverse alcoholic liver injury. Apigenin (Fig. 1, 4′, 5, 7-trihydroxyflavone) is a natural flavonoid compound widely distributed in a variety of fruits, vegetables, and medicinal plants [16,17], and its some beneficial effects have been reported, such as antioxidation [18], anti-inflammation [19], and antitumor effects [20]. Recent literature data have also indicated that apigenin may exert a protective effect on liver from some chemical reagents, such as acetaminophen, furan, and N-nitrosodiethylamine [21–23], and the mechanisms may be related to the increment of hepatic antioxidant ability. However, the protective effect of apigenin on alcohol-induced liver injury has not been reported. In this study, our aim was to investigate the effect and possible molecular mechanisms.

2. Materials and methods

2.1. Chemicals and reagents

Apigenin was kindly provided by Suzhou Baozetang Medical Technology Co., Ltd. (Suzhou, China), suspended in 0.5% sodium carboxymethyl cellulose solution, and the purity of the natural product was >98% as determined by high performance liquid chromatography. Gluthion (reduced glutathione sodium) was procured from Pharminvest SPA (Italy) and dissolved in normal saline. Erguotou wine was a product of Beijing Red Star Co., Ltd. (Beijing, China), the composition of the wine is alcohol and water (56/44, v/v). The assay kits for reduced glutathione (GSH), glutathione peroxidase (GSH-PX), glutathione reductase (GR), glutathione S-transferase (GST), and malondialdehyde (MDA) were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The ELISA kit for mouse TNF-α was purchased from Shanghai Xitang Bio-technological Co., Ltd. (Shanghai, China). Anti-CYP2E1 antibody was obtained from Proteintech Group Co., Ltd. (Wuhan, China). Anti-NF-κB p65 and anti-β-actin antibodies were purchased from Cell Signaling Technology (Boston, MA, USA). Anti-PPARα, anti-DGAT, anti-SREBP-1c, anti-FAS, and anti-CPT-1 antibodies were purchased from Abcam Company (Cambridge, UK). All other reagents used in this study were of analytical grade.

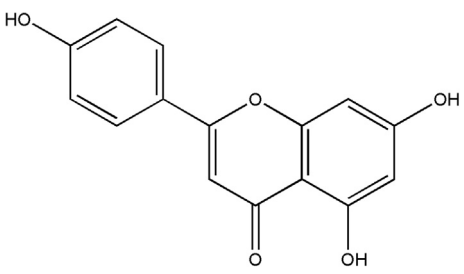

Fig. 1. Structure of apigenin.

2.2. Animals and experimental protocols

Kunming mice (male, 20 ± 2 g) were purchased form Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and maintained in a room controlled temperature (22–24 °C) and humidity (55–60%). These mice were allowed free access to food and water and acclimated to the laboratory environment for 3 days prior to the study. The animal study was approved by the University Ethics Committee and conducted according to the regulations for the Use and Care of Experimental Animals at Soochow University.

These experimental mice were randomized into 5 groups, namely control group, model (alcoholic liver injury) group, apigenin 150 and 300 mg/kg groups, and gluthion 240 mg/kg group. The medicine-treated mice were given apigenin orally by gavage or gluthion by tail intravenous injection in the morning (9:00–10:00) for 30 days, and the control and model mice were orally given an equivalent volume of 0.5% sodium carboxymethyl cellulose solution. Except for the control mice, the alcoholic liver injury was simultaneously induced by orally feeding 56% erguotou wine by gavage in the afternoon (14:00–15:00) for 30 days, and the daily amount of the wine administered to the mice was gradually increased from 0.10 ml/10 g body weight to 0.15 ml/10 g body weight within one week according to animal tolerance. Finally, all of the mice were sacrificed by stunning and cervical dislocation, and livers were collected for parameter measurements.

2.3. Measurements of hepatic GSH, GSH-PX, GR, GST, MDA, and TNF-α levels

Hepatic GSH, GSH-PX, GR, GST, and MDA levels were determined as described by Yang et al. [21]. For the hepatic TNF-α measurement, partial hepatic tissues were immediately put into ice-cold normal saline containing 50 U/ml aprotinin. The tissue homogenate (10%, w/v) was prepared and then centrifuged at 1200 × *g* for 10 min, the supernatant obtained was used for TNF-α measurement according to the ELISA method following the manufacturer's instructions on a VersaMax plate reader (Molecular Devices, CA, USA).

2.4. Histological observation

Liver specimens of mice were fixed in 10% formaldehyde solution and embedded in paraffin for hematoxylin and eosin (HE) staining, and then examined under a light microscope. The degree of hepatic steatosis was graded as described by Zhang et al. [24].

2.5. Western blot analysis for protein expression

Hepatic protein was extracted using a commercial kit (Keygen Biotech, Nanjing, China) according to the manufacturer's instructions and the protein concentration was determined using a bicinchoninic acid kit (Beyotime Institute of Biotechnology, Jiangsu, China). Western blot assay was performed as described by Cui et al. [25]. In brief, equal amounts of protein (50–70 μg) from each sample were loaded on 10% SDS-polyacrylamide gel and separated by electrophoresis. The protein bands obtained were then transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). Afterwards, the membranes were blocked with 5% skimmed milk at room temperature for 1.5 h and then incubated with respective primary antibodies of CYP2E1 (1:500 dilution), NF-κB p65 (1:600 dilution), PPARα (1:500 dilution), DGAT (1:1000 dilution), SREBP-1c (1:200 dilution), FAS (1:1000 dilution), CPT-1 (1:300 dilution), and β-actin (1:1000 dilution) at 4 °C overnight. Next, the membranes were washed and incubated with fluorescent secondary antibody at room temperature for 1 h. The protein blots were densitometrically