



Paeonol, the main active principles of *Paeonia moutan*, ameliorates alcoholic steatohepatitis in mice

Shilian Hu^{*}, Gan Shen, Weigang Zhao, Feng Wang, Xiaodong Jiang, Dabing Huang

Department of Geriatrics, Anhui Evidence-based Medicine Center, Anhui Geriatrics Institute, Anhui Provincial Hospital, Hefei 230001, China

ARTICLE INFO

Article history:

Received 7 April 2009

Received in revised form 27 August 2009

Accepted 28 December 2009

Available online 4 January 2010

Keywords:

Paeonol
Alcoholic liver injury
Inflammation
Apoptosis

ABSTRACT

Aim of study: Paeonol, a major phenolic component of Moutan Cortex, is traditionally used as a Chinese herbal medicine in various diseases including hepatitis. Evidence shows that paeonol has anti-inflammatory, anti-tumor, and anti-atherosclerosis effects. However, the effect of paeonol on alcoholic liver injury remains obscure. The present investigation was designed to determine the effects of paeonol on alcohol-induced hepatic injury in mice.

Materials and methods: The degree of alcoholic liver injury was evaluated biochemically by measuring serum markers and pathological examination. Real-time PCR and ELISA methods were used to check the expression of cytokines. Western blotting was used to check CYP 450 expression.

Results: Treatment with paeonol significantly attenuated the level of serum aminotransferase, reduced the severe extent of hepatic cell damage, steatosis, and the infiltration of inflammatory cells in a model of alcoholic liver injury ($P < 0.05$). Interestingly, paeonol markedly decreased hepatic mRNA expression of lipogenic genes ($P < 0.05$) while had no effect on protein expression of hepatic CYP2E1. Furthermore, paeonol significantly decreased serum and tissue inflammatory cytokine levels, tissue lipid peroxidation, neutrophil infiltration and inhibited the apoptosis of hepatocytes ($P < 0.05$). Kupffer cells isolated from ethanol-fed mice produced high amounts of tumor necrosis factor alpha, whereas Kupffer cells from paeonol treatment ethanol-fed mice produced less tumor necrosis factor alpha ($P < 0.05$).

Conclusions: These findings suggest that paeonol may represent a novel, protective strategy against alcoholic liver injury by attenuating hepatic steatosis, inflammatory response and apoptosis.

© 2009 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Alcohol-induced liver disease progresses from early steatosis, inflammation, necrosis, to fibrosis/cirrhosis and/or hepatocellular carcinoma (Williams, 2006). Factors that link alcohol intake to the onset and progression of hepatic damage still are poorly defined. It has been shown that liver P450 enzyme promotes hepatic lipid accumulation, which is considered the first step in the development of alcoholic liver disease (ALD) (Isayama et al., 2003; Kessova et al., 2003; Cederbaum, 2006; Lu and Cederbaum, 2008). Furthermore, progression beyond hepatic steatosis, an important step of second hit is the increased inflammation and cell death (Vidali et al., 2008). These mechanisms include the activation of intrahepatic macrophages, also known as Kupffer cells, releasing proinflammatory cytokines, chemokines and reactive oxygen species (ROS), as well as complex interactions between alcohol metabolism, various

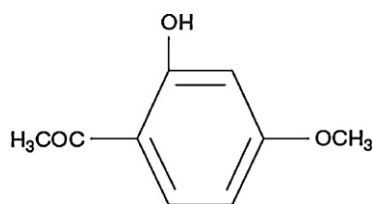
hepatic cells, multiple cytokines, and the immune system (Hoek and Pastorino, 2002; Nagy, 2003; Arteel, 2003, 2008; Albano, 2006; Dey and Cederbaum, 2006). Studies using animal models suggest that alcohol intake permeabilizes the gut, resulting in increased hepatic endotoxin levels, which is followed by activation of Kupffer cells. Activated Kupffer cells generate oxidative stress and produce a variety of soluble factors and cytokines (Yin et al., 1999). Among them, tumor necrosis factor alpha (TNF- α) is considered as one of the most important cytokines involved in the pathogenesis of alcoholic liver injury (Yin et al., 1999; Ji et al., 2004), while interleukin-6 (IL-6) has been shown to protect against alcoholic liver injury (Hong et al., 2002, 2004).

Due to thousands of years of experience, Chinese herbal medicines are considered as a rich source of new therapeutic agents. Many compounds with new structural features and mechanisms of actions have been isolated from herbal medicines. Natural products are potential sources of novel anti-hepatitis drugs that may be applicable to liver disease therapy. Paeonol, a micromolecular phenolic compound (Scheme 1) (Mimura and Baba, 1981), is the main component of a Chinese herbal medicine which prepared from the root bark of *Paeonia moutan* and isolated from the herb *Pycnostelma paniculatum* K.S. and the root of the plant *Paeo-*

^{*} Corresponding author at: Department of Geriatrics, The Affiliated Provincial Hospital of Anhui Medical University, Hefei 230001, Anhui, China.

Tel.: +86 551 2283867; fax: +86 551 2283875.

E-mail address: hushilian@yahoo.cn (S. Hu).



Scheme 1. Structure of paeonol (2-hydroxy-4-methoxyacetophenone).

nia suffruticosa Andrew. Paeonol is used as a food additive and in traditional oriental medicines in treating various diseases and inflammatory diseases including hepatitis (Wu, 1966; Zhang et al., 1996). Paeonol was later identified to have various pharmacological and physiological effects such as anti-inflammation, anti-bacteria, immuno-regulation, and anti-tumor effects (Wu, 1966; Eun et al., 2006; Ishiguro et al., 2006; Bu et al., 2008; Chunhu et al., 2008; Lee et al., 2008; Sun et al., 2008; Tsai et al., 2008; Zhong et al., 2009; Li et al., 2009). The most interestingly pharmacologic property of paeonol is anti-inflammatory and anti-atherosclerosis effects, suggesting that paeonol maybe have a protective effect on ALD (Chou, 2003; Ishiguro et al., 2006; Li et al., 2009). However, the effects of paeonol on alcoholic liver injury and its related mechanisms remain obscure. The present investigation was designed to determine the effects of paeonol on alcohol-induced hepatic injury in mice. Furthermore, this study was undertaken to unravel the possible mechanism responsible for the protective properties of paeonol on alcohol-induced liver injury. Our findings suggest that paeonol may represent a novel drug to prevent alcoholic liver injury by attenuating steatosis, inflammatory response, and apoptosis. The results of this investigation may provide a scientific explanation for the traditional application of this herbal medicine in liver disease therapy.

2. Materials and methods

2.1. Drugs and chemicals

Paeonol was purchased from Tianshi Pharmaceutical Factory of Tongling (Cat. No. 010521, 10 mg/2 ml) (Tongling, Anhui, China). RPMI 1640, Hepes buffer, collagenase IV, Pronase E, Dnase, Nyco-denz and LPS from *Escherichia coli* were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals used in these experiments were analytical grade from commercial sources.

2.2. Animals

Eight to ten-week-old healthy Kunming male mice were provided by the Department of Animal Sciences, Anhui Medical University and kept at constant temperature ($20 \pm 1^\circ\text{C}$) under a 12 h light/12 h dark cycle from 6:00 am to 18:00 pm. They were housed in plastic cages with free access to food and water. All procedures followed the guidelines for humane treatment of animals set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Animal experiments and treatment with drugs

Eight to ten-week-old male mice were pair-fed Lieber DeCarli liquid diet containing 5% ethanol or a control diet in which ethanol was substituted isocalorically replaced by dextrin maltose (Bio Serv Inc., French town, NJ) for up to 8 weeks as described previously (Jeong et al., 2008). Ethanol was introduced gradually by increasing the content by 1% (v/v) every day until the mice were consuming diets containing 5% (v/v) ethanol for up to 8 weeks. During the entire

feeding period, the mice of model group and the mice treated with different doses of paeonol were observed to consume similar volumes of ethanol diets daily and their body weight gains were also similar.

Five groups of mice (8–10 each), were separated as follows: (1) pair-fed untreated mice; (2) model alcohol feeding mice; (3–5) model mice treated with different doses of paeonol (100, 200 and 400 mg/kg, respectively). In the treatment groups, different drugs were administered orally by gastric intubation every day for 8 weeks. The mice in normal and model control groups were given the same volume of vehicle only.

2.4. Blood chemistry

Serum alanine transaminase (ALT), triglycerides, and cholesterol were determined using a clinical chemistry analyzer system (PROCHEM-V; Drew Scientific, Barrow-in-Furness, UK).

2.5. Liver histology analysis

Formalin-fixed liver tissue was processed for staining with H&E and then examined under the light microscope (Olympus IX70, Japan). For analysis of fat accumulation in the liver, 10- μm thick frozen sections were stained with Oil Red O (Vector Laboratories, Burlingame, CA). The assessment was done by an experienced pathologist, who was unaware of treatment conditions.

2.6. The TUNEL and myeloperoxidase (MPO) assay

Apoptotic hepatocytes in sections of mouse liver tissue were detected using an *in situ* apoptosis detection kit (Promega) as instructed by the manufacturer. Neutrophils were immunostained with anti-MPO polyclonal antibodies (ZYMED Laboratories, San Francisco, CA). Immunohistochemical staining for MPO was performed according to the standard streptavidin-peroxidase method described in the procedure program of streptavidin-peroxidase reagents kit (Sigma, St. Louis, MO).

2.7. Western blotting

Western blot analyses were performed with proteins from liver homogenates (50 μg) using anti-CYP2E1 (1:1000 dilution) polyclonal antibody. Immunoreactive bands were visualized on nitrocellulose membranes using alkaline-phosphatase-linked anti-mouse or rabbit antibody and the ECF detection system with a PhosphorImager (GE Healthcare, Piscataway, NJ). The densities of bands were quantified by using an ImageQuant software (GE Healthcare). The relative density was calculated by the ratio of the interest gene density/ β -actin gene density.

2.8. Kupffer cells isolation and culture

Kupffer cells were isolated by *in situ* collagenase perfusion and differential centrifugation on OptiPrep (Sigma) density gradient as described previously (Horiguchi et al., 2008). Briefly, mouse livers were perfused *in situ* first with EGTA solution, followed by perfusion buffer, and digestion buffer at 37°C for 20–30 min. To remove hepatocytes, the homogenate was filtered and centrifuged at $25 \times g$ for 5 min at room temperature. The supernatant was transferred to a new tube and centrifuged at $400 \times g$ for 10 min at 4°C , while the pellet was resuspended in 6 ml of 17% OptiPrep, and loaded carefully with 3 ml of GBSS washing buffer and centrifuged at $1600 \times g$ for 17 min at 4°C . The cellular fraction recovered from the GBSS and 17% OptiPrep interphase was gently aspirated, mixed with 13 ml GBSS, and centrifuged at $576 \times g$ for 10 min at 4°C . Pellets

Download English Version:

<https://daneshyari.com/en/article/2546640>

Download Persian Version:

<https://daneshyari.com/article/2546640>

[Daneshyari.com](https://daneshyari.com)