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Salvianolic acid B protects against chronic alcoholic liver injury via SIRT1-mediated inhibition of CRP and ChREBP in rats

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HIGHLIGHTS

- This is the first report demonstrating that SalB ameliorates ALD in rats.
- SalB alleviates ALD involved SIRT1-mediated inhibition of CRP and ChREBP.
- HNF-1α is involved in SIRT1-mediated inhibition of CRP expression.

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ABSTRACT

Salvianolic acid B (SalB), a water-soluble polyphenol extracted from *Radix Salvia miltiorrhiza,* has been reported to possess many pharmacological activities. This study investigated the hepatoprotective effects of SalB in chronic alcoholic liver disease (ALD) and explored the related signaling mechanisms. In vivo, SalB treatment significantly attenuated ethanol-induced liver injury by blocking the elevation of serum aminotransferase activities and markedly decreased hepatic lipid accumulation by reducing serum and liver triglyceride (TG) and total cholesterol (TC) levels. Moreover, SalB treatment ameliorated ethanol-induced hepatic inflammation by decreasing the levels of hepatotoxic cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Importantly, SalB pretreatment significantly increased the expression of SIRT1 and downregulated the expression of inflammatory mediator C-reactive protein (CRP) and lipoprotein carbohydrate response element-binding protein (ChREBP). In vitro, SalB significantly reversed ethanol-induced down-regulation of SIRT1 and increased CRP and ChREBP expression. Interestingly, the effects of SalB on SIRT1, CRP and ChREBP were mostly abolished by treatment with either SIRT1 siRNA or EX527, a specific inhibitor of SIRT1, indicating that SalB decreased CRP and ChREBP expression by activating SIRT1. SalB exerted anti-steatotic and anti-inflammatory effects against alcoholic liver injury by inducing SIRT1-mediated inhibition of CRP and ChREBP expression.

1. Introduction

Alcohol abuse is common worldwide and has been recognized as a major cause of chronic alcoholic liver disease (ALD), which has become one of the most significant health problems in recent decades due to its high morbidity and mortality (Liangpunsakul and Crabb, 2016; Rehm et al., 2013). ALD encompasses a disease spectrum ranging from minimal abnormalities, such as steatosis, to more severe liver disease associated with inflammation, including alcoholic hepatitis (AH), advanced fibrosis, and cirrhosis (Pavlov et al., 2016; Szabo, 2015; Thiele et al., 2016). Inflammation, which is caused by a "second hit" combined with lipid accumulation, the "first hit" that primarily triggers steatosis, plays a critical role in the pathogenesis of ALD (Gao et al., 2016; Mantena et al., 2008). Therefore, a thorough understanding of the mechanisms that regulate hepatic steatosis and inflammation may be clinically relevant for preventing and treating ALD.

In recent years, the use of plant extracts and poly-herbal formulations to treat various liver diseases has been documented

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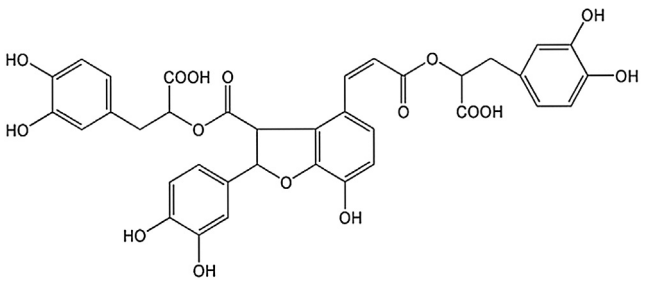

Fig. 1. Chemical structure of salvianolic acid B.

in various traditional systems of medicine (Jadeja et al., 2014; Rodriguez-Ramiro et al., 2016). Salvianolic acid B (SalB) (Fig. 1) is a major water-soluble component extracted from *Radix Salvia miltiorrhiza* and has been widely used for treating many types of illnesses, including hepatic, lung and renal diseases (Li et al., 2014; Yu et al., 2015). SalB has beneficial effects against hepatic fibrosis in animal models and has been shown to possess cardioprotective and neuroprotective activity via anti-oxidative and anti-inflammatory actions (Lee et al., 2013; Tang et al., 2016). Our previous studies have found that SalB plays a role in both acute ethanol-induced liver injury and non-alcoholic fatty liver disease (Li et al., 2014; Zeng et al., 2015). However, the role of SalB in preventing the onset and progression of chronic alcoholic liver injury remains unknown.

SalB is a natural compound that activates mammalian sirtuins 1 (SIRT1), an NAD-dependent class III histone deacetylase (HDAC) that plays important roles in several physiological processes, including gene transcription, senescence, energy metabolism, oxidative stress and inflammation (Li et al., 2014; Lv et al., 2015; Zeng et al., 2015). Hepatic nuclear factor-1α (HNF-1α) is a homeodomain transcription factor that interacts with a complex network of transcription factors to regulate gene expression in the liver and kidney as well as in pancreatic β-cells (Nishikawa et al., 2015; Shih et al., 2001). It has been reported that HNF-1α binds directly to the promoter of the gene encoding C-reactive protein (CRP) to modulate its expression (Grimm et al., 2011; Kyithar et al., 2013). CRP is primarily synthesized in the liver and is involved in many chronic diseases (Zhao et al., 2016). CRP levels correlate closely with changes in inflammation/disease activity, radiological damage and progression, and functional disability. Along with its role as an inflammatory marker, CRP also promotes inflammation through complement activation (Warren et al., 2015; Wiese et al., 2016). Previous studies have demonstrated that SIRT1 inhibits HNF-1α-mediated transcriptional activation of the CRP promoter by deacetylating lysine 16 of histone H4 around proximal HNF-1α binding sites in response to nutrient restriction (Grimm et al., 2011). Because inflammation has been recognized as a vital causative factor in the development of ALD (Mantena et al., 2008), we hypothesized that targeting the SIRT1/CRP pathway with SalB may represent a potential anti-inflammatory therapy for ALD.

Carbohydrate response element-binding protein (ChREBP), a glucose-responsive transcription factor, has an important role in ALD (Liangpunsakul et al., 2013). Mice carrying a liver-specific SIRT1 null mutation were shown to exhibit increased ChREBP expression and liver steatosis (Wang et al., 2010). Our recent study found that carnosic acid (CA) alleviates chronic alcoholic liver injury by regulating the SIRT1/ChREBP pathway in rats (Gao et al., 2016). CA and SalB share similar chemical structures with their phenolic hydroxyl groups, which may lead to similar pharmacological activities. However, it is unknown whether SalB exerts its protective effects against ALD through the SIRT1/ChREBP pathway.

The purposes of the present study were to explore whether SalB has beneficial effects against ALD and, if so, whether SalB exerts these protective effects against ALD through targeting SIRT1-mediated CRP and ChREBP inhibition.

2. Material and methods

2.1. Animal treatment and experimental design

Male Sprague-Dawley (SD, SCXK 2008-0002) rats weighing 180–220 g were obtained from the Animal Center of Dalian Medical University (Dalian, China). All animal procedures were performed according to the guidelines of the Institutional Animal Ethics Committee and were approved by the Institutional Animal Committee of Dalian Medical University. The rats were housed under standard laboratory conditions for approximately one week before experimentation. Fifty rats were randomly separated into five groups: 1) control, 2) control + SalB (30 mg/kg/d), 3) ethanol, 4) ethanol + SalB (15 mg/kg/d), and 5) ethanol + SalB (30 mg/kg/d). The doses of SalB were determined based on our preliminary previous study (Zeng et al., 2015)with modifications. Liquid diets were based on the Lieber-DeCarli formulation, and the ethanol content in the liquid diet was gradually increased from 5% in the first six weeks to 8% in the final two weeks (Gao et al., 2016). All the liquid diets were freshly prepared before distribution. The SalB groups received an intragastric administration every day, whereas the control group was treated with an equal volume of saline. After eight weeks, all the rats were euthanized, and blood and liver tissues were harvested.

2.2. Reagents

SalB (purity >98%) and resveratrol (RES) (98% purity) were purchased from Shanghai Winherb Medical Science Co., Ltd (Shanghai, China) and dissolved in distilled water for in vivo rat treatments and in vitro cell testing. Ethanol (purity >99%) and Ex527 (purity >98%) were obtained from Sigma Co., Ltd. (Sigma, USA). MEM and fetal bovine serum (FBS) are Invitrogen products that were purchased from Life Technologies (Carlsbad, CA, USA). Lipofectamine 3000 was purchased from Invitrogen (Karlsruhe, Germany).

2.3. Biochemical assays

The levels of triglyceride (TG) and total cholesterol (TC) in the liver as well as the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alcohol dehydrogenase (ADH), TG and TC in the serum were determined using commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Blood levels of ethanol were measured using a kit from BioVision Inc. (San Francisco, USA). All of the procedures were carried out according to the manufacturers' instructions.

2.4. Liver histopathological study

The middle lobe of the right liver was excised for histopathology. Liver tissues were cut into 3-mm-thick slices and fixed in 4% neutral buffered formalin for 24 h, paraffin embedded, sliced into 5 μm sections, and stained with hematoxylin-eosin (H&E) for histopathological examination.

2.5. Oil Red O staining

To visualize hepatic lipid accumulation, rat livers were removed and immediately snap-frozen at −70 °C. Then, 6-μm-thick cryostat sections were prepared on an APES-coated glass slide. Each section was washed with distilled water and then stained with Oil Red O