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Research Paper

Scutellarin protects against the liver injury induced by diosbulbin B in mice and its mechanism

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ABSTRACT

Ethnopharmacological relevance: Diosbulbin B (DB) is the main hepatotoxic compound distributed in *Dioscorea bulbifera* L., which is widely used for the treatment of cancer and thyroid disorders in Asia. Scutellarin (SC) is the main compound in medicinal herb *Scutellaria barbata* D. Don, which is usually combined with *Dioscorea bulbifera* used for cancer therapy in clinic.

Aim of the study: This study aims to investigate the protection of SC against the liver injury induced by DB and its engaged mechanism. In addition, the anti-tumor effect of DB and SC is further observed *in vivo*.

Materials and methods: The protection of SC against DB-induced liver injury was evaluated by detecting serum alanine/aspartate aminotransferases (ALT/AST) and alkaline phosphatase (ALP) activities, and further liver histological observation. The inflammatory response was assessed by detecting liver myeloperoxidase (MPO) activity, and serum levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interferon-γ (IFN-γ). Western-blot analysis was used to detect the protein expression. The oxidative liver injury was evaluated by detecting liver malondialdehyde (MDA) and glutathione (GSH) contents, and glutathione peroxidase (GPx) enzymatic activity. *In vivo* anti-tumor activity was analyzed in S180 tumor-bearing mice.

Results: SC significantly decreased the increased serum ALT/AST, and ALP activities induced by DB. Liver histological observation evidenced the protection of SC against DB-induced liver injury. SC obviously reduced the increased liver MPO activity and the number of MPO-positive staining cells induced by DB. SC also reversed the decreased expression of inhibitor of κB (IκB) and the translocation of nuclear factor κB (NF-κB) p65 from cytoplasm to nucleus induced by DB. In addition, SC significantly abrogated the increased serum levels of TNF-α, IL-6, and IFN-γ induced by DB. SC decreased the increased liver MDA content induced by DB significantly, and it also increased liver GSH level. The decreased GPx protein expression and its enzymatic activity induced by DB were both obviously reversed after SC treatment. The results in S180 tumor-bearing mice showed that SC combined with DB significantly inhibited tumor growth *in vivo*.

Conclusions: Our results demonstrate that SC prevents DB-induced liver injury by attenuating NF-κB-mediated hepatic inflammation and ameliorating liver oxidative stress injury. Meanwhile, DB plus SC has significant anti-tumor activity *in vivo*. This study indicates the potential combination of DB with SC for the treatment of cancer in clinic.

1. Introduction

Diosbulbin B (DB), a diterpene lactone, is the main compound isolated from *Dioscorea bulbifera* Linn (*Dioscoreaceae*), which is traditionally used to treat thyroid disease and cancer in China and some other Asia countries (Gao et al., 2002; Murray et al., 1984). Our previous study has already shown that DB is the major anti-tumor compound in *Dioscorea bulbifera* and exerts obvious

Abbreviations: DB, diosbulbin B; SC, scutellarin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; MPO, myeloperoxidase; TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; IFN-γ, interferon-γ; IκB, inhibitor of kappa B; NF-κB, nuclear factor κB; MDA, malondialdehyde; GSH, glutathione; GPx, glutathione peroxidase; DTNB, 5,5'-Dithio-bis(2-nitrobenzoic acid); ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin–eosin; LPO, lipid peroxidation; EF, ethyl acetate fraction of *Dioscorea bulbifera*; SE, ethanol extract of *Scutellaria barbata*

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anti-cancer effect *in vivo* (Wang et al., 2012). Although *Dioscorea bulbifera* is found to have strong anti-cancer effect during the clinical application, its hepatotoxicity also attracts the attention of people. Reports from our lab and other groups demonstrated that *Dioscorea bulbifera* induced serious liver injury both in animal experiments and in clinical practice (Liu, 2002; Wang et al., 2010, 2011; Su et al., 2012; Sheng et al., 2014). In addition, our previous studies also demonstrated that DB was the main hepatotoxic compound in *Dioscorea bulbifera*, and DB can induce liver oxidative stress injury (Wang et al., 2011; Ma et al., 2014). The reports about the hepatotoxicity induced by *Dioscorea bulbifera* and DB seriously hinder the application of *Dioscorea bulbifera* and the potential development of DB for cancer treatment in clinic.

Scutellarin (SC) is the main flavonoid contained in medicinal herb *Scutellaria barbata* D. Don (*Lamiaceae*), and it is also the chemical marker used by the Chinese Pharmacopoeia for evaluating the quality of *Scutellaria barbata* (Chinese Pharmacopoeia Commission, 2010). *Scutellaria barbata* is commonly combined with *Dioscorea bulbifera* used in numerous Chinese medical formulas for cancer therapy in clinic. In our previous study, we have found the protection of ethanol extract of *Scutellaria barbata* (SE) against the liver injury induced by ethyl acetate fraction of *Dioscorea bulbifera* (EF) (data not shown). Previous studies showed that SC had obvious anti-cancer effect *in vivo* and *in vitro* (Chan et al., 2009; Li et al., 2013a, 2013b). Meanwhile, there are various reports which demonstrate that SC exerts well protection against oxidative damage and inflammatory injury (Liu et al., 2003; Tan et al., 2010; Wang et al., 2011; Chen et al., 2013; Guo et al., 2013). In addition, SC was reported to attenuate concanavalin A-, selenium-, and brain ischemia/reperfusion-induced liver injury (Yang et al., 2003; Eltayeb et al., 2004; Tan et al., 2007). Thus, whether SC will protect against the hepatotoxicity induced by DB attracts our attention.

In the present study, we investigated the protection of SC against the hepatotoxicity induced by DB, and further explored the underlying mechanisms from inhibiting inflammatory and oxidative liver injury. Meanwhile, the anti-tumor activity of DB plus SC was analyzed *in vivo*.

2. Materials and methods

2.1. Drugs and reagents

Diosbulbin B (DB) (Fig. 1A) and scutellarin (SC) (Fig. 1B) were purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). ALT, AST, ALP, MDA, and MPO kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). 2-vinylpyridine, glutathione reductase, and 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits for TNF-a, IL-6 and IFN-γ were purchased from RapidBio (West Hills, CA). Antibodies against IκB, NF-κB p65, β-actin and lamin B were all purchased from Cell Signaling Technology (Danvers, MA). Antibody against GPx-1/2 was purchased from Santa Cruze (Santa Cruze, CA). Peroxidase-conjugated goat anti-Rabbit IgG (H+L) was purchased from Jackson ImmunoResearch (West Grove, PA). Nitrocellulose membranes were purchased from Bio-Rad (Hercules, CA). Enhanced chemiluminescence detection system was purchased from Millipore Corporation (Billerica, MA). NE-PER® nuclear and cytoplasmic extraction reagents were purchased from ThermoFisher Scientific (Waltham, MA).

2.2. Experimental animals

Specific pathogen free male ICR mice (18–22 g body weight) were purchased from Shanghai Laboratory Animal Center of Chinese Academy of Science (Shanghai, China). The mice were fed with a standard laboratory diet, given free access to tap water, and kept in a controlled room temperature (22 ± 1 °C), humidity (65 ± 5%), and a 12:12-h light/dark cycle. All mice received humane care in compliance with the institutional animal care guidelines approved by the Experimental Animal Ethical Committee of Shanghai University of Traditional Chinese Medicine (Approved number: 13005).

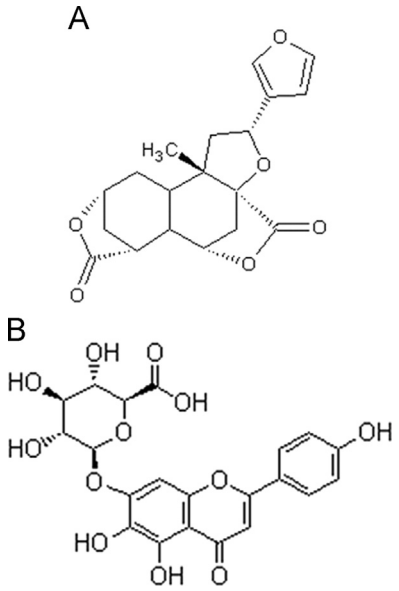

Fig. 1. Chemical structures of (A) diosbulbin B (DB) and (B) scutellarin (SC).

2.3. Treatment protocol for the evaluation of hepatotoxicity

Animals were divided into 4 groups: (1) vehicle control, (2) DB (300 mg/kg), (3) DB (300 mg/kg)+SC (7.5 mg/kg), and (4) DB (300 mg/kg)+SC (15 mg/kg). Mice were pretreated with SC at the dose of 7.5 mg/kg and 15 mg/kg for five days, and intragastric (i.g.) administration of DB (300 mg/kg, suspended in 0.5% CMC-Na) was done two hours after the last SC treatment. Twenty-four hours later, blood samples were collected by extirpating the eyeball and mice were sacrificed, and then their livers were obtained for further research.

2.4. Serum biochemical analysis

The blood samples obtained were kept at room temperature for 2 h. Serum was then collected after centrifugation at 840*g* for 15 min. Serum ALT, AST and ALP were measured with kits according to the manufacture's instructions.

2.5. Histological observations

Liver samples were fixed in 10% formalin and then embedded in paraffin. 5 μm thick paraffin sections were obtained and stained with hematoxylin–eosin (H&E) for histological observation.

2.6. Measurement of liver lipid peroxidation (LPO)

The amount of MDA, the end product of LPO, was determined according to the manufacturer's instruction. The tissue protein concentrations were measured according to the method of Bradford. Liver MDA amount was calculated based on protein concentrations of samples and expressed as nmol/mg protein.