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Protective role of liriodendrin in mice with dextran sulphate sodium-induced ulcerative colitis

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ABSTRACT

Sargentodoxa cuneata, containing syringaresinol and its glycoside liriodendrin as the main bioactive compounds, is a well-known traditional Chinese medicine for treating intestinal inflammation. In our preliminary study, liriodendrin inhibited NF-kB activation in sepsis-induced acute lung injury. The present study was designed to investigate its effect on dextran sulfate sodium (DSS)-induced colitis in a mouse model and to explore the possible related mechanisms. Experimental colitis was established by giving mice drinking water containing 3% (w/v) DSS for 7 days. The mice were pretreated with liriodendrin (100 mg/kg/day, intragastrically) 3 days before DSS treatment. We determined the effects of liriodendrin on disease activity index (DAI), colon length, histopathological examination, antioxidants, and anti-inflammatory activities. Our results showed that liriodendrin greatly decreased MPO and MDA activities and significantly increased SOD and GPx activities in the colon. Moreover, liriodendrin improved DAI, colon length and histological damage in colon and reduced the levels of pro-inflammatory cytokines, such as TNF-a, IL-1β and IL-6. Meanwhile, assessments by western blot revealed that liriodendrin significantly suppressed the activation of Akt and NF-κB pathways and up-regulated the expression of ERβ in the colon. In vitro, liriodendrin down-regulated production of pro-inflammatory cytokines and suppressed NF-κB signalling pathways in LPS-induced RAW 264.7 macrophages in a concentration-dependent manner. In addition, syringaresinol, the hydrolysate of liriodendrin, more potently down-regulated production of pro-inflammatory cytokines and suppressed NF-κB and Akt signalling pathways in LPS-induced RAW 264.7 macrophages,which were abolished by using a pure ER antagonist, ICI182, 780. Taken together, liriodendrin-mediated suppression of inflammatory damage in the colon may be attributable to the in vivo transformation to syringaresinol and liriodendrin may be a promising therapeutic approach preventive agent for colitis treatment.

1. Introduction

Ulcerative colitis (UC) is one of the two main forms of inflammatory bowel disease (IBD) in humans. UC is characterized by recurrent remission and relapse, involving the mucosa of colorectum [1]. In North America, the incidence of ulcerative colitis ranges from 2.2 to 14.3 cases per 100,000 persons per year [2]. Numerous studies have demonstrated that uncontrolled immune system activation and oxidative stress damage are considered to play crucial roles in the pathophysiology of IBD. Recent studies have illustrated that Akt pathway acts as a key downstream signaling component that regulates several inflammatory in colitis and associated colon cancer [3,4]. Nuclear factor-kappa B (NF-κB) also plays an important role in immune dysregulation in the pathogenesis of ulcerative colitis [5]. Therefore, limiting the inflammatory is thought to be an effective therapeutic strategy for ameliorating IBD. Another critical possible etiologic factor in the pathogenesis of intestinal damage has been extensive focus on reactive oxygen species (ROS) [6]. The imbalance between prooxidant and antioxidant mechanisms is a promising therapeutic strategy in IBD. In addition, recent studies documented that low endogenous antioxidant

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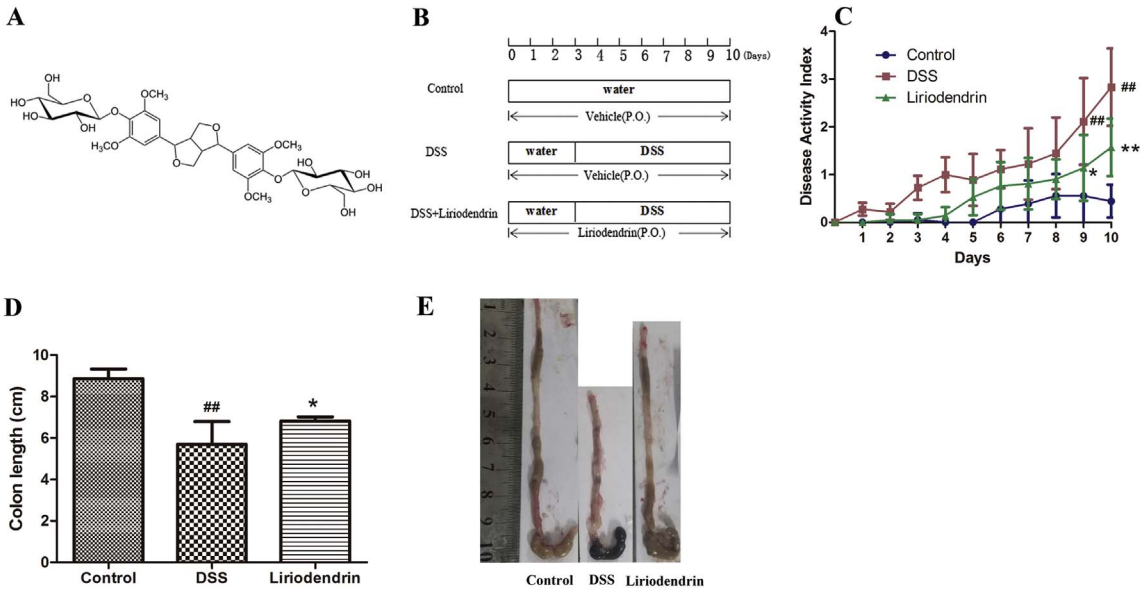

Fig. 1. Liriodendrin protected mice against DSS- induced acute colitis.
(A) The chemical structure of liriodendrin. Liriodendrin attenuated DSS-induced colitis in mice. (B) Animal model of DSS-induced colitis in mice. (C) The disease activity index changes were detected daily. (D and E) colon length was measured after 7 days of DSS administration. Te values presented are the mean ± S.E.M (n = 8 in each group). ## $P < 0.01$compared with the control group, ** $P < 0.01$ compared with the DSS group. * $P < 0.05$ compared with the DSS group.

defenses such as superoxide dismutases (SOD) and glutathione peroxidase (GPx) are implicated in the intestinal damage [7]. There are several anti-inflammatory and antioxidative drugs successfully used for ulcerative colitis. The medical therapies for UC mainly include 5-aminosalicylic acid, corticosteroids, immunosuppressor and biological agent [8,9]. But those medical therapies result in many adverse events and poor treatment responses. Therefore, exploring novel medicines may provide new therapies to UC.

Extensive ongoing research efforts have been invested in developing traditional Chinese medicine for treating ulcerative colitis, due to the abundant resources and more safer use in human. Sargentodoxa Cuneata is a well-known traditional Chinese medicine for treating abdominal inflammation, such as acute appendicitis, rheumatic arthritis, and ulcers, however, the bioactive compounds responsible for these protective effects remain unknown. We have determinated four active ingredients in Sargentodoxa Cuneata by HPLC and purified liriodendrin from Sargentodoxa Cuneata by macroporous resin [10,11]. Liriodendrin, (chemical structure shown in Fig. 1A) a type of lignins, possesses many beneficial effects, including anti-arrhythmic, anti-myocardial ischemia, anti-inflammatory and antioxidant [12–14]. Previous reports have shown that liriodendrin inhibited AP-1 and NF-κB activities in human synovial sarcoma SW982 cells [15]. Recently, we demonstrated that pretreatment with liriodendrin prevented sepsis-induced acute lung injury by up-regulating the expression of SIRT1 [16]. Furthermore, liriodendrin protected indomethacin-induced gastric ulcer by inhibiting H + /K + -ATPase [17]. However, whether liriodendrin exerts protective effect on the ulcerative colitis, either in animal or human, has not been reported.

Herein, we investigate the antioxidant and anti-inflammatory activities of liriodendrin with DSS-induced colitis and its possible related mechanisms, especially as related to the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), myeloperoxidase (MPO) and cytokine levels. Furthermore, liriodendrin treatment up-regulated the expression of ERβ in the colon. To further assess its anti-inflammatory effect, we hydrolyzed liriodendrin with cellulose and performed in vitro experiments with RAW264.7 macrophages. We found that both liriodendrin and syringaresinol down-regulated production of pro-inflammatory cytokines by suppressing the activation of NF-κB pathways. Syringaresinol can also suppress the activation of Akt pathways by up-regulating the expression of ERβ in RAW264.7 macrophages.

2. Materials and methods

2.1. Drug and reagents

Liriodendrin was isolated from Sargentodoxa Cuneata as previously described [11]. The purity of liriodendrin was determined to be over 97% by normalization of the peak areas by HPLC and its structure was elucidated by the NMR. Dextran Sulphate Sodium (DSS) (M.W. = 36,000–50,000) was purchased from MP Biomedicals (Santa Ana, CA, USA). ELISA kit for detecting murine IL-6 was supplied by R & D Systems (Minneapolis, MN, USA). The myeloperoxidase (MPO), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) kits were obtained from Nanjing Jiancheng Bioengineering Institute (Jiangsu Province, China). All of antibodies for western blot were purchased from Cell Signaling technology (Danvers, MA, USA). BCA Protein assay reagent kit was supplied by Thermo Scientific (Waltham, MA, USA).

2.2. Cell culture

RAW264.7 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI 1640 medium supplemented with 100 U/ml streptomycin, 100 U/ml penicillin, and 10% (v/v) FBS. Cells (approximately 1×10^6 cells/ml) were seeded in 6-well plates before being subjected to treatments. Liriodendrin or syringaresinol was added 2 h before LPS (from *Escherichia coli*, Sigma) stimulation.

2.3. Animals

6-week-old male BALB/c mice (20 ± 2 g) were supplied by Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All of the mice were housed with a 12 h/12 h light-dark cycle in a controlled sterile environment maintained at 22 ± 2 °C. The mice had free access to water and commercial chow for at least one week to adjust themselves to the environment before the experiments. All the animal