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Magnolol treatment attenuates dextran sulphate sodium-induced murine experimental colitis by regulating inflammation and mucosal damage

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ABSTRACT

Magnolol, the main and active ingredient of the Magnolia officinalis, has been widely used in traditional prescription to the human disorders. Magnolol has been proved to have several pharmacological properties including anti-bacterial, anti-oxidant and anti-inflammatory activities. However, the effects of magnolol on ulcerative colitis (UC) have not been reported. The aim of this study was to investigate the protective effects and mechanisms of magnolol on dextran sulphate sodium (DSS)-induced colitis in mice. The results showed that magnolol significantly alleviated DSS-induced body weight loss, disease activities index (DAI), colon length shortening and colonic pathological damage. In addition, magnolol restrained the expression of TNF-α, IL-1β and IL-12 via the regulation of nuclear factor-κB (NF-κB) and Peroxisome proliferator-activated receptor-γ (PPAR-γ) pathways. Magnolol also enhanced the expression of ZO-1 and occludin in DSS-induced mice colonic tissues. These results showed that magnolol played protective effects on DSS-induced colitis and may be an alternative therapeutic reagent for colitis treatment.

1. Introduction

Ulcerative colitis (UC), a form of inflammatory bowel disease (IBD), is a type of chronic and relapsing inflammatory of the gastrointestinal tract. Nowadays, millions of patients around the world are suffering with UC, and the risk of colon cancer is also significantly increasing [1,2]. Although multiple factors such as environmental changes, gene variations, gut microbiota, and immunological stimulus are thought to be associated with UC, its etiology and pathogenesis are complicated and remain uncertain [3]. To study this disease, a model of mice colitis has been used by the oral administration of dextran sulphate sodium (DSS), which could research into the pathogenesis of UC and is similar to human UC [4].

Emerging evidences show that the intestinal barrier plays a vital role in intestinal inflammation in experimental colitis [5]. In addition, it has been reported that the damage of intestinal epithelial barrier is regulated by the abnormal activity of some pro-inflammatory signals. Specifically, nuclear factor-κB (NF-κB), a transcription factor, promotes transcription of genes encoding pro-inflammatory cytokines. Its activation induces the production of important immune mediators, such as TNF-α, IL-1β and IL-12 [6]. The activation of NF-κB has been observed in UC patients and DSS-induced colitis mice [7]. Moreover, Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of the nuclear hormone receptor family. Its activation could regulate NF-κB activation which decreases the levels of pro-inflammatory cytokines [8,9]. Currently, many therapeutic drugs have been used for UC, but most of these drugs exists many resistances problem, and the therapies for UC have major adverse effects. Therefore, the development of novel therapies for UC is urgently needed.

Magnolol (Fig. 1), the main and active ingredient of the Magnolia officinalis, is a traditional Chinese medicine herb which has been widely used in traditional prescription to the human disorders. Magnolol has been proved to have several pharmacological effects including antibacterial, anti-oxidant and anti-inflammatory activities [10]. Previous studies in our laboratory have confirmed that magnolol inhibits the inflammatory response in mouse mastitis and acute lung injury [11,12]. Furthermore, magnolol also has been widely used for treatment of gastrointestinal disorders [13], indicating it may have the potential to be become an available anti-UC agent. Thus, we investigated the potential protective effects and mechanisms of magnolol on DSS induced UC model mice.

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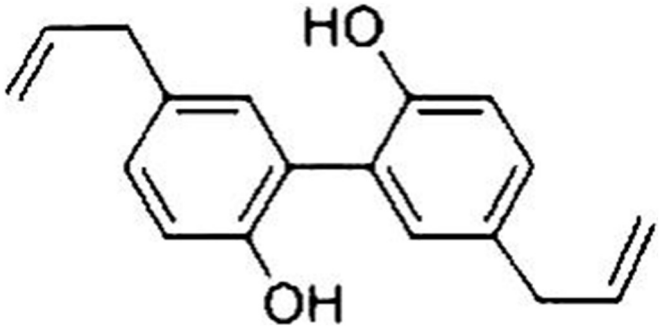

Fig. 1. The chemical structure of magnolol.

2. Materials and methods

2.1. Chemicals

Magnolol (> 98% HPLC) was purchased from (Chengdu, China). DSS (molecular weight 36–50 kDa) was obtained from MP Bio medicals, Morgan Irvine, CA. Mouse TNF-α, IL-1β and IL-12 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Biolegend (San Diego, CA, USA). Rabbit monoclonal antibodies IκB, p65, p-IκB, and p-p65 were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). The primary antibody that was raised against zonula occludens-1 (ZO-1) and occludin were obtained from Wanleibio Co., Ltd. (Liaoning, China). Rabbit monoclonal antibody PPAR-γ was purchased from GeneTex (Shanghai, China). β-actin and horseradish peroxidase conjugated goat anti-rabbit antibodies were provided by Tianjin Sungene Biotech Co., Ltd. (Tianjin, China).

2.2. Animals and mice model of DSS-induced colitis

Male C57BL/6 mice, weighing approximately 21 to 23 g, were obtained from the Center of Experimental Animals of Bethune Medical College of Jilin University (Jilin, China). All mice were housed in a temperature maintained room (24 ± 1 °C). Before experimentation, all mice were adapted to their new environment for a minimum of 1 week, and supplied with standard diet and tap water ad libitum. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Mice were randomly divided into six groups of six mice each:

Group I: Control group

Group II: DSS group

Group III: magnolol (25 mg/kg body weight) + DSS group

Group IV: magnolol (50 mg/kg body weight) + DSS group

Group V: magnolol (100 mg/kg body weight) + DSS group

Group VI: magnolol (100 mg/kg body weight) group.

For acute colitis model, the DSS group mice were exposed to 2.5% DSS, which was dissolved in drinking water, continuously for 5 days. In the magnolol + DSS groups, the mice were treated with different doses of magnolol 3 days before and during DSS treatment via oral gavage once per day [14]. Mice in the control group and DSS group were given the same volume of water. On the 5 days of the colitis induced by DSS, mice were sacrificed, samples were collected, and the colon was excised from cecum to 1 cm above the anus. The lengths of the colon were detected, which indirectly stipulated the inflammatory index of the colon.

2.3. Measurements of UC and clinical colitis scoring

The baseline clinical disease activity index (DAI) was the sum evaluation of the clinical score [15]. In brief, body weight, feces condition and fecal blood test scores were added together. Body weight was measured on a daily basis. The disease activity index (DAI) was calculated with a previously established scoring system [15].

2.4. Myeloperoxidase (MPO) assay

MPO is an enzyme that is directly proportional to the number of neutrophils in the tissue. The MPO activity was measured according to the manufacturer's instructions. Briefly, colitis tissue, weighing approximately 100 mg, was fixed with phosphate buffered saline (PBS, weight/volume ratio 1:9) and homogenized. The supernatants were analyzed using the MPO kit (Jiancheng biotechnology, China) and detected by spectrophotometer at an absorbance value of 460 nm.

2.5. Histologic analysis

For the histological analysis, day 5 after colitis induction with 2.5% DSS, the colon was washed in PBS. Then, the colonic tissues were fixed in 10% formalin. The colon specimens were embedded in paraffin and then deparaffinized with xylene and rehydrated for hematoxylin & eosin (H&E). Histological grading was assessed according to a scoring scheme in the H&E-stained sections [16].

2.6. Preparation of cecal bacterial lysates

Cecal bacterial lysates (CBLs) were prepared as described by Dieleman et al. [17]. Briefly, the cecal contents in each group were solubilized by vortexing the contents in Roswell Park Memorial Institute (RPMI) 1640 medium and then incubating them with 10 μg/ml DNase and 0.01 M $MgCl_2$. Then, the contents were homogenized for 3 min using 0.1 mm glass beads. The homogenate was centrifuged at 10000*g* for 10 min. The supernatant was filtered through a 0.45 μm syringe filter.

2.7. Mesenteric lymph node cell cultures

Mesenteric lymph node (MLN) was harvested from mice of six experimental groups. Single cell suspensions were prepared as described by Ruyssers [18]. Approximately 4×10^5 MLN cells and 20 μg/ml CBL were cultured in RPMI-1640 medium with 10% fetal bovine serum and 50 mg/ml gentamicin at 37 °C with 5% CO_2 for 72 h. The culture media was then collected for cytokine analysis.

2.8. Cytokine analysis by ELISA

The colonic tissues were weighed and homogenized with PBS [1:9 (w/v)] on ice and then centrifuged at 2000*g* for 40 min at 4 °C. The supernatant was collected and stored at −20 °C. The TNF-α, IL-1β and IL-12 levels were detected by ELISA kits according to the manufacturer's protocol.

2.9. RNA isolation and real-time PCR

Total RNA from colonic tissues were extracted using TRIzol (Invitrogen, Carlsbad, CA) as per manufacturer's instructions. The RNA was reverse transcribed into cDNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo). The quantification of relative mRNA concentrations, including TNF-α, IL-1β, IL-12 and β-actin mRNA, was performed with qRT-PCR using a 7500 Fast Real-Time PCR System (Applied Biosystems) and the SYBR Green Plus reagent kit (Roche), as described elsewhere [19]. The sequences of primers are listed in Table 1.

2.10. Western blot

The colonic tissues were homogenated, then centrifuged for 10 min at 4 °C. The supernatant was collected and its protein concentration was