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Perspective

Patchouli alcohol ameliorates dextran sodium sulfate-induced experimental colitis and suppresses tryptophan catabolism

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ABSTRACT

Despite the increased morbidity of ulcerative colitis (UC) in recent years, available treatments remain unsatisfactory. *Pogostemon cablin* has been widely applied to treat a variety of gastrointestinal disorders in clinic for centuries, in which patchouli alcohol (PA, $C_{15}H_{26}O$) has been identified as the major active component. This study attempted to determine the bioactivity of PA on dextran sulfate sodium (DSS)-induced mice colitis and clarify the mechanism of action. Acute colitis was induced in mice by 3% DSS for 7 days. The mice were then given PA (10, 20 and 40 mg/kg) or sulfasalazine (SASP, 200 mg/kg) as positive control via oral administration for 7 days. At the end of study, animals were sacrificed and samples were collected for pathological and other analysis. In addition, a metabolite profiling and a targeted metabolite analysis, based on the Ultra-Performance Liquid Chromatography coupled with mass spectrometry (UPLC–MS) approach, were performed to characterize the metabolic changes in plasma. The results revealed that PA significantly reduced the disease activity index (DAI) and ameliorated the colonic injury of DSS mice. The levels of colonic MPO and cytokines involving TNF-α, IFN-γ, IL-1β, IL-6, IL-4 and IL-10 also declined. Furthermore, PA improved the intestinal epithelial barrier by enhancing the level of colonic expression of the tight junction (TJ) proteins, for instance ZO-1, ZO-2, claudin-1 and occludin, and by elevating the levels of mucin-1 and mucin-2 mRNA. The study also demonstrated that PA inhibited the DSS-induced cell death signaling by modulating the apoptosis related Bax and Bcl-2 proteins and down-regulating the necroptosis related RIP3 and MLKL proteins. By comparison, up-regulation of IDO-1 and TPH-1 protein expression in DSS group was suppressed by PA, which was in line with the declined levels of kynurenine (Kyn) and 5-hydroxytryptophan (5-HTP) in plasma. The therapeutic effect of PA was evidently reduced when Kyn was given to mice. In summary, the study successfully demonstrated that PA ameliorated DSS-induced mice acute colitis by suppressing inflammation, maintaining the integrity of intestinal epithelial barrier, inhibiting cell death signaling, and suppressing tryptophan catabolism. The results provided valuable information and guidance for using PA in treatment of UC.

Abbreviations: DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; MPO, myeloperoxidase; TJs, tight junctions; RIP3, receptor interacting protein 3; MLKL, mixed lineage kinase domain-like; UPLC–MS, ultra-performance liquid chromatography coupled with mass spectrometry; OPLS-DA, orthogonal projections to latent structures discriminant analysis; PCA, principal components analysis; VIP, variable importance in projection; 5-HT, 5-Hydroxy tryptamine; 5-HTP, 5-Hydroxy tryptophan; IDO-1, indoleamine 2, 3-dioxygenase-1; TPH-1, tryptophan hydroxylase-1.

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1. Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing intestinal disease, which mainly includes the subtypes of ulcerative colitis (UC) and Crohn's disease (CD) [1]. Unlike CD, inflammation in UC is limited in the colon and rectum, affecting mainly the mucosal layer of the intestinal wall [2]. Multiple factors, for instance, defective immune response, mucosal barrier dysfunction, dysbiosis of commensal microbiota and genetic susceptibility are considered to contribute to the etiology of UC [2]. According to the literature, activation of immunocytes secreting multiple cytokines stimulates an inflammatory response [2,3], and increased cell death signals resulting in breakdown of the intestinal epithelial barrier perpetuates chronic intestinal inflammation [4,5].

The morbidity of UC increased over the past years [6–9], however, the conventional therapies either chemical drugs or biological applications are not able to offer satisfactory treatment of UC. Severe adverse reactions, for example infection and lymphoma, as well as low efficacy are often observed [10,11]. *Pogostemon cablin* (Blanco) Benth has been applied in treatment of gastrointestinal disorders such as diarrhea and peptic ulcer for very long time in China [12,13], which aqueous extract was reported to protect intestinal barrier [14]. Patchouli alcohol (PA, $C_{15}H_{26}O$), with a structure of sesquiterpene (Fig. 1), is identified and isolated as the major active component of *Pogostemon cablin*. PA processed multiple bioactivities, for example, anti-inflammation, anti-gastric ulcer and immune-modulation [15–18]. This makes PA a promising alternative agent for treatment of UC.

In this study, the effect of PA was investigated on a murine model of colitis induced by dextran sodium sulfate (DSS), a well-established model that mimics many clinical symptoms of human UC [19,20]. Sulfasalazine (SASP), a first line therapeutic medicine of IBD, was used as a positive control at the dose of 200 mg/kg to evaluate the efficacy of PA. The doses of PA 10, 20 and 40 mg/kg, are proximately 5, 10 and 20 fold of clinical dose, if converting based on the clinical dose of herbal material. PA was orally administrated for 7 days to DSS-challenged mice, and the weight changes, colon length, disease activity index (DAI) score and histopathological changes of colon were measured. In addition to that, levels of colonic cytokines and mucin RNAs were determined. The expression of tight junction proteins and proteins encoding cell death signaling were also measured. Metabolic changes associated with UC were illustrated by metabolic profiling and targeted metabolite analysis. The metabolomic results were validated by detecting alteration of critical enzymes and an in vitro confirmatory experiment. The results showed that PA ameliorated DSS-induced mice acute colitis by suppressing inflammation, protecting intestinal epithelial barrier and suppressing tryptophan catabolism. This may suggest that PA has the potential to be developed as a promising agent for treatment of UC.

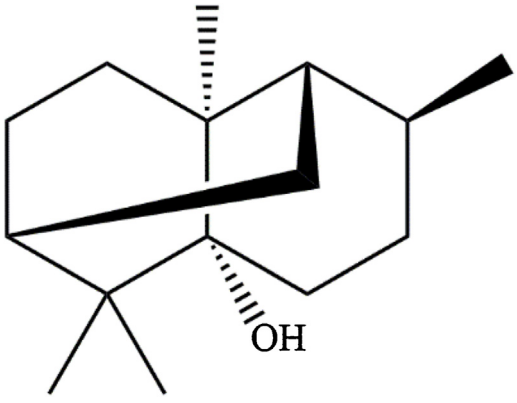

Fig. 1. Chemical structure of patchouli alcohol.

2. Materials and methods

2.1. Preparation of chemicals and reagents

PA was isolated from patchouli oil in the laboratory and its purity (99.0%) was confirmed by gas chromatographic analysis in our previous study [21]. Its structure (Fig. 1) was confirmed as described [18]. PA and SASP, the positive control, were dissolved in tween-80 water solution (2% wt/vol). The vehicle containing the identical proportion of tween-80 was administered to the control and DSS groups.

DSS was the product of MP Biomedicals (molecular weight: 36,000–50,000, MP Biomedicals, Canada). SASP was purchased from Shanghai Fuda Pharmaceutical Co. Ltd. Tween-80 was purchased from Sigma-Aldrich (St Louis, MO, USA). The BCA protein kit was purchased from Thermo Scientific Pierce. Myeloperoxidase (MPO) assay kit was obtained from Nanjing Jiancheng Biotechnology Company. Mouse TNF-α (Lot#: 4281407), IFN-γ (Lot#: E09468-1660), IL-1β (Lot#: E09327-1648), IL-6 (Lot#: E09362-1656), IL-4 (Lot#: E09342-1642), IL-10 (Lot#: E17772-111), IL-12 (Lot#: E09401-1645) and IL-17 (Lot#: E09521-1647) ELISA kits were the products of eBioscience (CA, USA). Primary antibodies against mouse ZO-1 (sc-8146), ZO-2 (sc-11448), claudin-1 (sc-17658), occludin (sc-8145), receptor interacting protein 3 (RIP3, sc-135170) and β-actin (sc-130656) antibodies were purchased from Santa Cruz Biotechnology (CA, USA). Anti-mouse Bax (2772s) and Bcl-2 (2876s) antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Monoclonal antibody MLKL (MABC604) was purchased from Merck Millipore (CA, USA). Anti-IDO-1 (NBP1-49632) and anti-TPH-1 (NB300-176) were purchased from Novus Biologicals (Littleton, USA). All other chemicals used in this study were of analytical grade.

2.2. Animals

Seven to eight week old male BalB/C mice, weighed 22–24 g, were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine (GZUCM, Guangzhou, China) with the approval number of 44005800002727. Animals were housed under standard conditions (temperature 23 ± 2 °C, humidity 50–70%, and12-h light/dark cycle) and fed with standard chew pellets and water *ad libitum*. Mice were acclimatized for 1 week before experiments. The handling of mice and all experimental procedures performed were approved by the Committee for Animal Care and Use at GZUCM and in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3. Induction of colitis

Experimental colitis was induced through oral intake of 3% (wt/vol) DSS in drinking water for 7 days. Throughout the experiment, the volumes of water or DSS-solution consumed in all groups were measured daily. The animals were randomly assigned into 6 groups (n = 16–20/group): control group, DSS group, DSS plus PA-treated (10, 20 and 40 mg/kg) groups, and DSS plus SASP (200 mg/kg) group. Mice in the control group were supplied with distilled water, whereas all other experimental groups were given 3% DSS. PA and SASP groups were orally administered with PA (at the doses of 10, 20 and 40 mg/kg, respectively) or SASP (200 mg/kg), while the mice in control and DSS groups received equal volumes of vehicle from day 1 to 7, respectively. The dose of PA was selected based on previous reports and our pilot study. The body weight,