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Therapeutic roles of polysaccharides from *Dendrobium Officinaleon* colitis and its underlying mechanisms

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ABSTRACT

Polysaccharide, as a promising candidate to meet the medication requirement of ulcerative colitis (UC), is increasingly attracting extensive interest. *Dendrobium officinale* has been widely used to treat gastrointestinal sickness in the clinical treatment of Traditional Chinese Medicine. However, it remains largely unknown whether polysaccharides (DOPS) from *Dendrobium officinale* can treat UC. The purpose of this paper is to confirm therapeutic action of DOPS to UC and explored its underlying mechanisms. We noted that DOPS could dramatically improve clinical signs and symptoms, decrease mortality, alleviate colonic pathological damage, and reestablish the balance of pro- and anti-inflammatory cytokines in DSS-induced acute UC mice. Moreover, DOPS treatment could also markedly suppress the activation of NLRP3 inflammasome and β-arrestin1 *in vivo* and *in vitro*. This study showed that DOPS possesses appreciable therapeutic effect to treat experimental acute UC mice. Its mechanism could be related to inhibition of NLRP3 inflammasome and β-arrestin1 signaling pathways.

1. Introduction

Ulcerative colitis (UC) is one of the typical inflammatory bowel diseases, resulting from chronic inflammation and sores in the lining of the rectum and large intestine (colon) (Ungaro, Mehandru, Allen, Peyrin-Biroulet, & Colombel, 2016). As a chronic disease, the repeated outbreak and protracted course is the typical feature of UC, which seriously influence life quality of patients, and is sometimes life-threatening (Marchioni & Kane, 2014). A number of studies have linked UC to an increased risk of cancerization of colitis (Porter, Tribble, Aliaga, Halvorson, & Riddle, 2008), and proved that 40% UC cases with more than 25 years ultimately developed colorectal cancer (Shivashankar, Tremaine, Harmsen, & Loftus, 2016). Surprisingly, UC has long been an intractable medical problem yet still not well solved since its discovery. Currently, the mainstay medication for the management of UC, such as anti-inflammatory drugs, immunosuppressants and antibiotics, can only help relieve disease symptoms (Peyrin-Biroulet et al., 2015; Talaei, Atyabi, Azhdarzadeh, & Saadatzadeh, 2013). Long-term use of these drugs could result in some serious side-effects, including steroid dependence and secondary infections (Kane, 2006). Therefore, it is urgent to develop new therapeutic medicines with high efficiency and low toxicity for UC treatment.

Plenty of studies revealed that the imbalance of pro- and anti-inflammatory cytokines caused by the immune system overreacting in warding off attacks may play a critical role in the occurrence and worsening of UC (Neurath, 2014; Strober & Fuss, 2011). The secretion of pro-/anti-inflammatory cytokines is regulated by multiple signal molecules, among which, much of the attention recently has recently been focused on an inflammasomes, especially the NLRP3 inflammasome (Bauer et al., 2010; Jiang, Zhong, Sun, & Rong, 2016). NLRP3 is the member of NOD-like receptor (NLR) family and expressed in immune cells and epithelial, which could be activated by numerous such as LPS and ATP. The NLRP3 inflammasome is a protein complex consisting of NLRP3 protein, the adaptor protein ASC and pro-caspase-1

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protein. Its activation can cleave ASC and caspase1 proteins. The dissociated and activated caspase1 can farther promote the secretion and maturation of pro-inflammatory cytokines such as IL-1β and IL-18 (Lazaridis et al., 2017), thereby breaking the delicate balance of pro- and anti-inflammatory cytokines. The abnormal expression of pro-inflammatory cytokines can damage the tight junctions between epithelial cells and alter intestinal permeability to trigger and aggravate UC through a pathological cascade process (Fiocchi, 1996; Soufli, Toumi, Rafa, & Touil-Boukoffa, 2016). Thus, the activation of NLRP3 inflammasome is perhaps the most vital step of UC deterioration. However, its activation mechanism remains elusive. β-arrestin proteins play important role in the G protein-coupled receptor (GPCR) regulation. β-arrestin 1 is one of the super-family members and expressed in many tissues and cells, which is involved in inflammation, tumor and autoimmune diseases. Encouragingly, the β-arrestin1, a multifunctional protein, has been identified and recognized as playing a role in activating NLRP3 inflammasome by Mao's group in a recent research (Mao et al., 2015). Therefore, NLRP3 inflammasome and β-arrestin1 signaling pathways may be potential therapeutic targets for UC treatment.

Polysaccharides have received widespread attention in recent years due to its remarkable pharmacological activities with fewer side effects. Currently, many polysaccharides exhibited striking therapeutic benefits for UC (Kaur, Gulati, & Singh, 2016; Lv et al., 2017), indicating that polysaccharides are promising candidates to meet the target of UC treatment. *Dendrobium officinale* (DO) is a precious traditional Chinese herbal medicine which belongs to *Dendrobium* species, *Orchidaceae*. The DO has very high medicinal and edible values called as “Gold in Herbs” and “Life-saving Mesona chinensis” (Silva and Ng, 2017). DO has been widely used to treat gastrointestinal sickness in China for one thousand years, which was the earliest documented in a famous materia medica book of the Eastern Han Dynasty, “Shennong's classic of materia medica” (Luo et al., 2017). Polysaccharides are regarded as the main components of DO. Over the past few years, the polysaccharides (DOPS) from *Dendrobium officinale* have been successfully extracted and purified (Hua, Zhang, Fu, Chen, & Chan, 2004). Their significant anti-inflammatory and intestinal immunomodulating benefits have also been validated (Lin, Shaw, Sze, Tong, & Zhang, 2011; Xie, Liu, Zhang, Zha, & Luo, 2016). Therefore, we speculated that DOPS may play a prophylactic and therapeutic role in supressing UC. To testify this hypothesis, here, we investigated the protective effects and potential mechanisms of DOPS on an acute UC mice model induced by DSS and LPS-stimulated NCM460 cells model.

2. Materials and methods

2.1. Drugs and reagents

DOPS was prepared by previous report Hua et al. (2004). Its structure was characterized by Hua et al. (2004) and shown in Fig. 1A. The total polysaccharide in DOPS was 93.80%. The FT-IR spectrogram showed that DOPS has intense and broad absorption peak around 3414 cm^{-1}, a weak absorption peak at 2924 cm^{-1} and 1324 cm^{-1}, and an asymmetrical extension at 1175 cm^{-1} (Supplementary Fig. 1A). They are allocated to O—H stretching vibrations, C—H stretching vibrations and C—C or C—O stretching vibrations, respectively. These are the characteristic absorption of polysaccharides. The monosaccharide composition of DOPS was mannose, glucose and arabinose with a molar ratio of 5.55:1:0.12 (Supplementary Fig. 1B). In addition, the average molecular weight of DOPS is 393.8 kDa (Supplementary Fig. 1C). All these characteristics of DOPS implied that the structure of DOPS was the same as Hua et al. (2004).

Dextran sodium sulfate (DSS) was obtained from MP Biomedicals (MW; 36000–50000, MP Biomedicals, Solon, OH, USA). The Bio-Plex mouse Cytokines Panel assay kits were purchased from Bio-Rad (Bio-Rad, USA). Human IL-1β and IL-18 ELISA kits were bought from Huamei (Cusabio Biotech Co. Ltd, China). RNAiso Plus reagent, PrimeScript™RT reagent kit and SYBR Green PCR Master Mix were provided by Takara (Takara, Japan). RNeasy mini kit was from Qiagen. Antibodies against IL-18, IL-1β, β-arrestin1, NLRP3, ASC, caspase1 and GAPDH were purchased from Abcam (Abcam, USA). RPMI Medium 1640 basic, fetal bovine serum (FBS) and phosphate buffer saline (PBS) were obtained from GIBCO Laboratories (Grand Island, NY, USA) and penicillin G/streptomycin, MTT, Dimethyl Sulphoxide (DMSO) and LPS were purchased from Sigma (St. Louis, MO, USA).

2.2. Experimental animals

Male BalB/c mice (6–8 weeks old, 20 ± 2 g) were obtained from the Laboratory Animal Services Center, Guangzhou University of Chinese Medicine (Guangzhou, China). All of the mice were kept in specific pathogen-free conditions where they received standard food and sterilized water ad libitum under constant temperature (20–25 °C) and humidity (65–70%) with a 12-h light/dark cycle. All mice studies were performed according to institutional and National Institutes of Health guidelines for humane animal use. Experimental protocols were approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine.

2.3. Dose determination of DOPS in animal experiments

In order to pick the optimum dosage of DOPS in animal experiments, we first calculated the human DOPS amounts equivalent to the clinical dosage of DO, basing on its extraction rate from DO. Human DOPS amounts, then, were converted into its animal dosage by conversion principle for drug dosage between humans and animals. On this basis, a preliminary experiment was used to comprehensively evaluate the safety and efficacy of these DOPS dosages. Finally, the dosages of DOPS in UC mice were determined to 50, 100 and 200 mg/kg.

2.4. Induction of experimental acute UC model and treatment

Actue colitis was induced by administering 4% dextran sodium sulfate (DSS, dissolved in drinking water) for 7 days. 60 mice were randomly divided into five groups with 12 mice in each group, named normal control group, DSS model group, DOPS groups (50, 100 and 200 mg/kg, p.o.). Mice in each group were managed as schematic diagram of Fig. 1B. Except for normal control group were given distilled water, other groups were only allowed to freely drink 4% DSS throughout experimental period. During DSS treatment, mice of both normal control group and DSS model group were simultaneously given distilled water (10 ml/kg, p.o.) once daily, while DOPS groups were administrated with 50, 100 and 200 mg/kg DOPS (10 ml/kg, p.o.) once daily, respectively. The body weight, stool consistency, food intake and water intake were evaluated daily. The average daily food intake/water intake was calculated as formula (1).

$$\text{Average daily food/ water intake} = \frac{Tf\ (\text{or}Tw)}{n} \quad (1)$$

where *Tf*, *Tw* and *n* were the total food intake weight, total water intake volume and number of animals, respectively. The experiment lasted for 7 days.

2.5. Observation of UC signs and symptoms

Body weight, feces status and bloody stools of all mice were observed and assigned a score everyday by an experimenter blinded to the protocol according to the standard scoring system (Ghia, Blennerhassett, Kumarondiveeran, Verdu, & Collins, 2006; Liang et al., 2017). On this basis, disease activity index (DAI) was calculated as formula (2). In addition, their food intake, water intake and mortality were also recorded every day in a double-blind manner.