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Regulation of JAK2/STAT3 and NF-κB signal transduction pathways; *Veronica polita* alleviates dextran sulfate sodium-induced murine colitis

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ABSTRACT

Ulcerative colitis (UC) is a major inflammatory bowel disease (IBD) has become a worldwide emergent disease. *Veronica polita* (VP) is a medicinal herb that has strong antioxidant and anti-inflammatory properties. In the present study, we studied the protective effect of VP on dextran sulfate sodium (DSS)-induced experimental colitis in mice. Phytochemical screening of VP extract demonstrated the presence of high total phenolic and flavonoid contents. Compared with the DSS group, VP significantly reduced clinical symptoms with less weight loss, bloody stool, shortening of the colon, and the severity of colitis was considerably inhibited as evidenced by the reduced disease activity index (DAI) and degree of histological damage in the colon and spleen. Also, treatment with VP considerably decreased the nitric oxide (NO) and malondialdehyde (MDA) level. VP remarkably downregulated the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), inducible nitric oxide synthetase (iNOS) and cyclooxygenase-2 (COX-2) in the colon tissue. Likewise, activation of the signal transducer and activator of transcription 3 (STAT3) and nuclear factor-kappa B (NF-κB) was effectively blocked by VP. Taken together, these results demonstrate that VP has an ameliorative effect on colonic inflammation mediated by modulation of oxidative stress and inflammatory mediators by suppressing the JAK2/STAT3 and NF-κB signaling pathways.

1. Introduction

Inflammatory bowel diseases (IBD) including ulcerative colitis (UC) and Crohn's disease (CD) have become an emergent and widespread health concern. UC is characterized by chronic and relapsing inflammation of the colon. Though the etiology of UC has not been clearly understood, it seems to involve damaged large intestinal epithelia, activated immune cells, and inflammatory factors in mucosa and submucosa [1]. Various animal models have been established to describe the complexity of UC pathogenesis, outlining basic molecular mechanisms and evaluating prospective human therapeutics [2]. A common mice model of colitis employs dextran sulfate sodium (DSS), a chemical colitogenic and anticoagulant. DSS-mediated intestinal inflammation is also not completely understood, but probably results from injury to the epithelial lining of the large intestine, permitting the propagation of pro-inflammatory cytokines [3].

Several factors are associated with the pathogenesis of UC, including the overproduction of reactive oxygen species (ROS), lipid peroxidation and nitric oxide (NO) in the inflamed colonic mucosal epithelium, immune response by mucosal inflammatory cytokines, and altered intestinal microbiota [4,5]. The study revealed that mucosal immunity activates during colitis and is accompanied by increased production of inflammatory cytokines and enzymes including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), inducible nitric oxide synthetase (iNOS), and cyclooxygenase-2 (COX-2) [6]. Elevated expression of cytokine activity is involved in inflammation and carcinogenesis. Several intracellular signaling pathways, comprising a signal transducer and activator of transcription 3 (STAT3) and nuclear factor-kappa B (NF-κB) activation, were proved to trigger colitis and controlled a multiple gene expression [7,8]. In normal physiological conditions, the p65/p50 NF-κB heterodimer remains inactive as a complex with an inhibitor of κB (IκBα). It becomes triggered in response to inflammatory stimuli via subsequent phosphorylation and degradation of IκBα [9] and thereby functionally activate NF-κB which translocate to the nucleus, where it controls the target gene expression. Besides, binding of cytokines with transmembrane receptors

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induces receptor dimerization of subunits and results in phosphorylation of Janus kinase (JAK) [10]. This phosphotyrosine on the cytokine receptor end then assists as a binding site for cytosolic STAT proteins and leads to a JAK-mediated tyrosine phosphorylation of STATs [11]. STAT3 has played a critical role in an autoimmune disease that induces excessive STAT3 activation, which initiates several autoimmune disorders [12]. Activation of STAT3 is dependent on its phosphorylation at the specific tyrosine residue (Y705), which facilitates the dimerization of STAT3. The activated STAT3 dimer then translocates into the nucleus to promote the transcription of target genes [13].

For a better understanding of the mechanisms involved in mucosal homeostasis and ulcerative colitis, animal models of mucosal inflammation are important. DSS-induced colitis in mice is used in preclinical studies for finding substances that may have therapeutic effects on IBD [14]. Phytomedicine has been significantly important for its ability to manage and treat inflammatory and immunomodulatory diseases [15]. *Veronica polita* (VP) is a plant of the plantaginaceae family that has been widely cultivated in Asia. In South Korea, VP was known as “gaeburlpool”. The major active compounds of VP extract such as apigenin-C-hexoside-C-hexoside, apigenin-C-hexoside-C-pentosides, isoscutellarein-Ohexoside-hexoside, isoscutellarein-hexoside, isoscutellarein-O-acetylhexosyl-hexoside, verminoside, luteolin-O-glucuronide, protocatechuic acid derivative II and grouped phenolic acids, phenylethanoids and phenolic compounds was determined by HPLC analysis [16]. Veronica species have become prominent due to their traditional uses and biological actions and has been reported for anti-inflammatory [17] and antioxidant [18] activities. Considering the traditional uses and biological activities, we hypothesized that VP may attenuate DSS-mediated ulcerative colitis by mitigating the oxidative stress, pro-inflammatory cytokines by regulating the JAK2/STAT3 and NF-κB signaling pathways.

2. Materials and methods

2.1. Chemicals and antibodies

DSS (36,000-50.000 M.W.) bought from MP Biomedicals (Illkirch-Graffenstaden, France). Protease inhibitor and HE staining reagents purchased from Sigma-Aldrich (St. Louis, MO, USA). RNA extraction kit (RiboEx and Hybrid-R) bought from Gene All (Seoul, South Korea). Griess reagent, cDNA synthesis kit (ReverTra Ace qPCR RT Kit), T-PER, and BCA protein assay kit purchased from Thermo Scientific (Waltham, Massachusetts, USA). SYBR Green qPCR Kit supplied from TOYOBO (Tokyo, Japan). TBARS assay kit obtained from Cayman (Ann Arbor, MI, USA). Primary antibodies COX-2, IL-1β, phospho-JAK2, phospho-STAT3, phospho-IκBα, phospho-NF-κB, and β-actin supplied by Cell Signaling (Danvers, MA, USA). Secondary antibody (goat anti-rabbit immunoglobulin g horseradish peroxidase) provided by Santa Cruz (CA, USA). WESTSAVE gold ECL detection kit obtained from Abfrontire (Seoul, South Korea). Zoletil 50 bought from Virbac (Carros, France).

2.2. Preparation of VP extract

The VP plant was authenticated based on its microscopic and macroscopic features by the Korean Institute of Oriental Medicine (KIOM). We prepared VP extract according to the previously described method [19]. Briefly, the plant was sliced and dried completely. The extract was prepared by maceration of the sample with 70% ethanol (twice for 2 h reflux), and then filtered extract was concentrated under vacuum centrifuge and dehydrated with a lyophilizer. The powder extract was liquefied in dimethyl sulfoxide (DMSO) and sterilized using a 0.22 μm syringe filter. The dried extract was kept at −20 °C. The study was conducted using a single batch of plant extract to avoid batch-to-batch variation and to maximize the product constancy.

2.3. Phytochemical analysis

Folin-Ciocalteu (FC) method was performed for phytochemical analysis of VP extract. Total phenolic and flavonoid contents of the VP extract were measured according to the previously designated method [20].

2.4. Experimental mice management and induction of colitis

Six-week-old C57BL/6 mice were handled in accordance with the animal welfare regulations of the Institutional Animal Care and Use Committee (IACUC; CBNU 2016-68) of the Chonbuk National University Laboratory Animal Center in South Korea. Mice were kept in standard mouse cages with a supply of *ad libitum* food and distilled water. Temperature (23 ± 2 °C), humidity (35–60%), and photoperiod cycle (12 h light and 12 h dark) were maintained during the experimental period. Mice were adapted to the laboratory conditions for 1 week before the experiment was started. For induction of colitis, we followed a stablished protocol [21]. Briefly, a total of 40 experimental mice were randomly divided into 4 groups: Group 1. Control mice received distilled water for 7 days; Group 2. Mice received DSS (5%) w/v in distilled water for 7 days; Group 3. Mice received DSS (5%) in distilled water and VP (200 mg/kg) orally for 7 days; Group 4. Mice received DSS 5% in distilled water orally and dexamethasone (1 mg/kg, intraperitoneally) for 7 days (Fig. 1a). At the end of the experimental period, mice were fasted overnight and anesthetized with Zoletil 50 (10 mg/kg). Samples were collected for experimental analysis.

2.5. Disease activity index (DAI) scoring

Mice weight and stool consistency were recorded daily for 1 week. DAI was scored according to the previously described method [22]. Briefly, for stool consistency, 0 points were given for normal stools, 2 points for pasty stools, and 4 points for liquid stools. Rectal bleeding was scored 0 points for no blood in haemoccult, 2 points for a positive haemoccult, and 4 points for gross bleeding. The sum of these individual scores gave a final mark, ranging from 0 for good clinical symptoms to 8 for severe colitis.

2.6. Histological analysis of colon and spleen

The colon and spleen were excised and gently rinsed with phosphate-*buffered* saline (PBS). Tissues obtained from the experimental mice were subjected to hematoxylin and eosin (H&E) staining. Specimens were fixed in 4% PFA for 24 h at 4 °C. Samples were processed using an auto processor (Excelsior ES, Thermo Scientific, USA). 5 μm sections were stained with H&E and mounted on glass slides. Digital imaging was done with a Leica DM2500 microscope (Leica Microsystems, Germany) at fixed 100× magnification. Histological scoring of colon and spleen tissue was assessed according to previously published method [23,24].

2.7. Analysis of lipid peroxidation and NO production

Malondialdehyde (MDA) and NO are important markers of oxidative stress. The colon tissue was homogenized and centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatant was collected and kept at −80 °C for analysis. We measured MDA and NO concentration in the colon tissue samples according to the commercial kit instructions.

2.8. RNA extraction and quantitative real-time polymerase chain reaction (qPCR)

The RNA was extracted from colon tissue according to the manufacturer’s instructions. The concentration of total RNA was quantified with the BioSpec-nano spectrophotometer (Shimadzu Biotech, Tokyo,