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Protective effect of decursin and decursinol angelate-rich *Angelica gigas* Nakai extract on dextran sulfate sodium-induced murine ulcerative colitis

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ABSTRACT

Objective: To investigate the anti-inflammatory effects of decursin and decursinol angelate-rich *Angelica gigas* Nakai (AGNE) on dextran sulfate sodium (DSS)-induced murine ulcerative colitis (UC).

Methods: The therapeutic effect of an AGNE was analyzed in a mouse model of UC induced by DSS. Disease activity index values were measured by clinical signs such as a weight loss, stool consistency, rectal bleeding and colon length. A histological analysis was performed using hematoxylin and eosin staining. Key inflammatory cytokines and mediators including IL-6, TNF-α, PGE_2, COX-2 and HIF-1α were assayed by enzyme-linked immunosorbent assay or western blotting.

Results: Treatment with the AGNE at 10, 20, and 40 mg/kg alleviated weight loss, decreased disease activity index scores, and reduced colon shortening in mice with DSS-induced UC. AGNE inhibited the production of IL-6 and TNF-α in serum and colon tissue. Moreover, AGNE suppressed the increased expression of COX-2 and HIF-1α and the increased production of PGE_2 in colon tissue were observed in mice with DSS-induced UC. Additionally, histological damage was also alleviated by AGNE treatment.

Conclusions: The findings of this study verified that AGNE significantly improves clinical symptoms and reduces the activity of various inflammatory mediators. These results indicate the AGNE has the therapeutic potential in mice with DSS-induced UC.

1. Introduction

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease, has been defined as relapsing inflammation in the digestive system. The course of IBD is unpredictable and its pathogenesis is not fully known [1]. UC, the chronic inflammation of the colon, is affected by factors including abnormal immune system of microbiota, a disorder of the cell wall caused by antibodies in the intestine, and or pathogenesis of epithelial cell of colon [2]. The primary symptoms of UC are abdominal pain, rectal bleeding, and diarrhea mixed with blood [3]. The wall of the colon consists of four sections: the mucosa, submucosa, muscular layer, and serous membrane. UC develops from pathological changes to the mucosa and submucosa, in which neutrophilic and eosinophilic leukocytes infiltrate the colon and cause the surrounding blood vessels and crypt abscess to become inflamed, causing ulcer formation [4]. Cytokines secreted from intestinal epithelial cells are important markers of the intestinal immune system, and regulate the inflammatory response in UC. Elevated levels of cytokines, including interleukin (IL)-6, IL-8, IL-12, and tumor necrosis factor-alpha (TNF-α), in serum and mucosal tissue samples have been reported in patients with IBD [5]. Cyclooxygenase (COX) has been implicated the progression of inflammation. Among them, COX-2 is not expressed under normal conditions in healthy tissues and cells, but is expressed under inflammation conditions. COX-2 is known to stimulate prostaglandins involved in the mediation of

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inflammation. In particular, prostaglandin E_2 (PGE_2) and COX-2 are expressed in the inflamed tissues of UC patients [6]. Hypoxia inducible factors (HIFs), which are hypoxia-mediated gene-regulating transcription factors, link inflammatory pathways. Among the HIFs, HIF-1 is used as a marker of inflammatory disease [7]. Expression of HIF-1α in the colon tissue of patients with UC has been reported to be elevated [8].

Therefore, the regulation of inflammatory cytokines and enzymes in the colon tissues is an important target for colitis treatment. Drugs containing aminosalicylates, such as sulfasalazine (SSZ) and mesalazine, are commonly used for IBD, but these drugs are associated with side effects, including gastrointestinal upset, headaches, and myocarditis [9]. Several phytonutrients that were used in traditional medicine demonstrated anti-inflammatory effects [10–12]. Of those studies, kolaviron, curcumin, and resveratrol were reported to prevent gastrointestinal mucosal damage [13–15]. Therefore, there has been growing interest in recent years in identifying herbal medicines that provide therapeutic benefits without undesirable side effects.

Angelica gigas Nakai (AGNE) is a Korean traditional herbal medicine and is one of the most popular herbal medicines used in Asian countries, including Korea, Japan (*Angelica acutiloba* (*A. acutiloba*)), and China (*Angelica sinensis*). AGNE has been studied extensively and found to contain various substances, including coumarins [16]. Coumarins comprise decursin and decursinol angelate (D/DA), which have been used as a traditional medicine for the treatment of anemia, as a sedative, and as an anodyne or a tonic agent. It was previously reported that the oral acute and subacute toxicity and the genotoxicity of extracts containing approximately 95% D/DA [17,18]. AGNE has been widely used for the treatment of dysmenorrhea, amenorrhea, menopausal syndromes, abdominal pain, injuries, migraine headaches, and arthritis [19,20]. AGNE is also known to exert anti-bacterial and anti-amnesic effects as well as to inhibit acetylcholinesterase, depress cardiac contraction, and activate protein kinase C [21,22]. In addition, D/DA from AGNE has been evaluated for anti-inflammatory effects [23–25]. The therapeutic effects of D/DA in colonic inflammation have not yet been documented, despite the reported anti-inflammatory effect of D/DA. IBD is usually assessed by using mice as animal models of dextran sulfate sodium (DSS)-induced UC. The IBD in these animal models is similar to acute UC in humans, thus, these models are frequently used in colitis research [26]. In the present study, the authors investigated the effects of AGNE on colonic inflammation in a mouse model of UC induced by DSS.

DSS-induced UC in mice is characterized by bloody stools, the mucosal infiltration of inflammatory cells, and ulceration [27]. Therefore, the aims of this study were: 1) to analyze the main bioactive components derived from AGNE; 2) to evaluate inhibition of inflammatory-gene expression of AGNE in mice with DSS-induced UC; 3) to examine clinical signs such as weight loss, reduction of colon length, diarrhea, and bloody feces.

2. Materials and methods

2.1. Animals and reagents

Male ICR mice (4 week old) were obtained from Daehan Biolink Animal Facility (Chungbuk, Korea). The mice were housed in a pathogen-free environment for at least 1 week to allow them to adapt to the environmental changes and were euthanized by using CO_2 inhalation at the end of the study. All animal studies were carried out in accordance with regulations issued by the Institutional Review Board of Daegu Haany University (confirmation number: DHU2016-045). DSS (molecular weight: 36 000–50 000) was purchased from MP Biomedicals (Solon, OH, USA). Anti-mouse TNF-α/IL-6, recombinant TNF-α/IL-6, and biotinylated TNF-α/IL-6 were purchased from BD Bioscience (San Diego, CA, USA). Specific antibodies against COX-2, HIF-1α, and β-actin were obtained from Abcam (Cambridge, UK) and Santa Cruz Biotechnology (Santa Cruz, CA, USA). SSZ and other chemical reagents were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Preparation and high performance liquid chromatography (HPLC) analysis of AGNE

Fresh AGNE was purchased from the Ginbu GAP Farming Corporation (Pyeongchang, Gangwon, Korea) in 2015. AGNE was air-dried and stored at room temperature. Dried AGNE (4 kg) was extracted with 20 L of 95% ethanol at 80 °C for 4 h in non-woven fabric. The extract was collected and filtered to remove precipitates. The filtrate was concentrated again and the 330 g of concentrate obtained was designated AGNE. The main components present in AGNE were analyzed using an Agilent HPLC system (1100 series, Agilent Technology, Santa Clara, CA, USA) with an Agilent Zorbax SB-C18 column (250.0 mm × 4.6 mm, 5 μm). Signals were detected at 329 nm using a UV monitor (1100 series, Photo-Diode Array UV/Vis detector, Agilent Technology). The D/DA contents of AGNE were quantified using a calibration curve established by injecting dilutions of each standard (62.5–250.0 μg/mL) into the HPLC system (correlation coefficient $\geq$ 0.996; standard curve formula: y = 20.928 28x + 38.319 67). Standards (D/DA) for HPLC analysis were obtained from Chengdu Biopurity Phytochemicals Ltd. (Chengdu, China).

2.3. Induction of UC by DSS and experimental procedures

UC in mice was induced by adding 5% (w/v) DSS to drinking water, which was provided *ad libitum* for 7 d. The mice were examined daily for weight loss, stool consistency, and the presence of gross bleeding. The mice were randomized into 5 groups (n = 7 per group). They received AGNE (10, 20, 40 mg/kg), SSZ (300 mg/kg) as a positive control, and saline as a negative control. AGNE and SSZ were diluted with saline (150 μL) and orally administered once daily starting at day 0 of DSS treatment. The mice were euthanized and assessed after 7 d of DSS administration [28].

2.4. Assessment of disease activity index (DAI)

DAI values were assessed by scoring clinical signs such as a weight loss, stool consistency, and rectal bleeding to evaluate the efficacy of AGNE on DSS-induced rat model [29,30]. In the present study, the values were calculated using the following formula: DAI = (weight loss score) + (stool consistency score) + (occult blood/gross bleeding score). Each variable was expressed in number from 0 to 4 depending on the three scores. Each DAI score was characterized as follows: for body