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Polydatin ameliorates dextran sulfate sodium-induced colitis by decreasing oxidative stress and apoptosis partially via Sonic hedgehog signaling pathway

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ABSTRACT

Background: Inflammation, oxidative stress and epithelial barrier dysfunction have been implicated in inflammatory bowel disease (IBD) pathology. The targeted inhibition of these features may represent a promising therapeutic strategy for IBD. Polydatin is an effective natural antioxidant that possesses strong antioxidant and anti-apoptotic properties. Thus, we studied the protective effects of polydatin treatments on a mouse model of experimental colitis.

Methods: Acute colitis was experimentally induced by adding 3% dextran sulfate sodium (DSS) to the drinking water provided to mice for 7 days and by administering different doses of polydatin (15, 30, or 45 mg/kg) during the same period. Mice were also treated with the Sonic hedgehog (Shh) pathway inhibitor cyclopamine to estimate the efficacy of polydatin and Shh inhibitors on colitis. The disease activity index (DAI), colon length, histology, levels of oxidative and apoptotic mediators and levels of Shh pathway components were evaluated.

Results: The polydatin treatment significantly attenuated the DAI, colon shortening and histological damage. In addition, polydatin administration effectively decreased malondialdehyde (MDA) levels and increased the activities of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Polydatin also inhibited apoptosis in mice with colitis by downregulating the expression of the pro-apoptotic proteins Bax, caspase 3 and cleaved caspase 3 and increasing the expression of the anti-apoptotic protein Bcl-2. Furthermore, polydatin modulated Shh signaling pathway activation. After polydatin treatment, the main components of the Shh pathway, including Shh, Patched (Ptc), Smoothened (Smo), and glioblastoma-1 (Gli1), were upregulated at the mRNA and protein levels. Blockade of the Shh pathway using cyclopamine abolished the effects of polydatin on mice with colitis.

Conclusion: Based on these observations, polydatin may suppress experimental colitis at least partially by regulating the Shh signaling pathway.

1. Introduction

Ulcerative colitis (UC) is a chronic, relapsing, nonspecific type of inflammatory bowel disease (IBD) that begins in young adulthood and continues throughout a person's lifetime. Over the past 50 years, the incidence of UC has been 120–200/100,000 persons [1]. Since the pathological mechanisms underlying UC remain largely unknown, environmental factors, individual susceptibility, immune imbalance and gut microecology have been investigated. In recent years, oxidative stress and epithelial barrier disruption [2,3] have been identified as essential intracellular events that contribute to UC. Hence, strategies targeting these features may represent promising therapeutic treatments for UC.

Sonic hedgehog (Shh), the most well-studied ligand of the hedgehog (Hh) signaling pathway, plays an important regulatory role in embryonic development and organ formation during early development [4] by guiding gut differentiation and maintaining intestinal homeostasis [5]. In addition, the Shh pathway regulates oxidative stress and epithelial cell apoptosis in the gastrointestinal (GI) tract [6]. Polydatin (3,4′,5-trihydroxy-3-β-D-glucopyranoside, PD) is a glucoside of resveratrol that is extracted from the rhizomes of *Polygonum cuspidatum* [7,8]. PD has a number of pharmacological activities, and the therapeutic potential of PD has been extensively studied. As shown in previous studies, PD plays an important pharmacological role in liver fibrosis

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Table 1
The histological score.

	0	1	2	3	4
Percent of tissue damage	No	≤25%	≤50%	≤75%	100%
Extent of tissue damage	No	Mucosa	Mucosa and submucosa	Beyond the submucosa	
Degree of inflammation	No	Slight	Moderate	Severe	
Extent of crypt damage	No	Basal 1/3	Basal 2/3	Only the surface epithelium was intact	The entire crypt and epithelium were lost

[9], ischemia-reperfusion injury [10], murine osteoarthritis [11] and acute small intestinal injury in hemorrhagic shock [12] by exerting anti-inflammatory, antioxidant, and anti-apoptotic effects. Therefore, we postulate that PD may exert these protective effects on IBD. In fact, Jun et al. [13] reported that PD suppresses inflammation and reduces nuclear factor-κB activation in an experimental colitis model. However, the other mechanisms underlying the effects of PD on colitis have not yet been completely elucidated. Therefore, this study was conducted to investigate, for the first time, the potent antioxidant and anti-apoptosis effects of PD on colitis, and the mechanisms underlying the therapeutic action of PD might be associated with the activation of the Shh signaling pathway.

2. Materials and methods

2.1. Animals and treatment

Six- to 8-week-old male C57BL/6 mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were housed in a laboratory with controlled conditions (21 ± 2 °C temperature and 50 ± 5% humidity) and were maintained on a 12-hour light/dark cycle. The mice had access to a standard laboratory diet and sterile water ad libitum during the entire experimental period. The animals were acclimated to the Experimental Animal Laboratory for 10 days before the study was initiated. All animal protocols used in this study were approved and experimental procedures were conducted strictly in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Reagents

Dextran sulfate sodium (DSS; MW 36,000–50,000 Da) was obtained from MP Biomedicals (Aurora, OH, USA). PD (purity > 98%) and cyclopamine (Cyc; purity ≥ 99%) were obtained from Beijing J&K Scientific Ltd. (Beijing, China) and Aladdin Industrial Corporation (Shanghai, China), respectively. The malondialdehyde (MDA) reagent kit was purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.3. Experimental design

Mice were randomly assigned into five groups ($n = 8$ mice per group): (1) the control group, (2) DSS-induced group, and (3)–(5) DSS-induced mice treated with PD (15, 30 or 45 mg/kg [14]). Colitis was induced in all groups except the control group by adding 3% DSS to the drinking water for 7 days. In the control and DSS-induced groups, mice were intraperitoneally injected with physiological saline, whereas the mice in the DSS + PD (15, 30 or 45 mg/kg) groups were intraperitoneally injected with PD suspended in physiological saline for 7 days. Five groups of mice ($n = 8$ mice per group) were used to evaluate the efficacy of an inhibitor of Shh signaling in mice with DSS-induced colitis: (1) the control group; (2) DSS group: mice with DSS-induced colitis; (3) PD group: mice with DSS-induced colitis that were treated with polydatin (30 mg/kg) (4) Cyc group: mice with DSS-induced colitis treated with cyclopamine (5 mg/kg); and (5) PD + Cyc group: mice with DSS-induced colitis treated with both polydatin (30 mg/kg) and cyclopamine (5 mg/kg [15]). PD and Cyc were initially dissolved in dimethyl sulfoxide (DMSO) and then diluted with physiological saline to a final concentration of 1% DMSO for intraperitoneally injection. All animals were sacrificed on day 8. During the course of the experiment, the body weight, stool consistency, and gross bleeding scores were evaluated for each animal to calculate the disease activity index (DAI) [16].

2.4. Histopathological assessment

Mice were sacrificed after the experiment, the colons were removed, and the colon length of each mouse was measured. Then, the dissected colon tissue was washed with cold phosphate-buffered saline (PBS). Part of the distal colon was cut and fixed with 4% paraformaldehyde for the histopathological analysis, and the remaining tissue was stored at −80 °C for biochemical examinations. The portion fixed with 4% paraformaldehyde was embedded in paraffin, stained with hematoxylin and eosin (H&E) and then analyzed in a blinded manner. The histological score was determined as described in Table 1 [17].

2.5. MDA assay

A commercial kit based on thiobarbituric acid (TBA) reactivity was used to determine the colonic contents of MDA, according to the manufacturer's protocols. We first mixed trichloroacetic acid with the colon tissue homogenate, centrifuged the sample to obtain the supernatant and then added TBA. The absorbance of the supernatant was measured at 532 nm by spectrophotometry [18].

2.6. Antioxidant enzyme measurements

Colon tissue was homogenized in cold saline. The levels of SOD and GSH-Px activities were determined using commercial kits according to the manufacturer's instructions. SOD activity was tested using the xanthine/xanthine oxidase method that is based on the production of O_2^- anions. The values are reported as U/mg protein. GSH-Px activity was assessed by examining the rate of NADPH degradation at 412 nm. The values are presented as U/g protein [19].

2.7. Real-time polymerase chain reaction (RT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA) was used to extract total RNA from the colon samples according to the manufacturer's protocol. The cDNA templates were produced from the extracted RNA using reverse transcriptase (Thermo Scientific, USA) according to the manufacturer's instructions. SYBR Green probes and an ABI 7500 system were used for RT-PCR. The mRNA expression level of each target gene was normalized to the β-actin level (Table 2).

2.8. Western blot analysis

Nuclear and cytosolic proteins were extracted from the colon tissues according to the manufacturer's protocol (Thermo Fisher Scientific Inc., USA). The protein extracts from each sample were boiled for 5 min, and