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Ginsenoside Rd attenuates the inflammatory response via modulating p38 and JNK signaling pathways in rats with TNBS-induced relapsing colitis

Xiao-Lai Yang [a,b], Tian-Kang Guo [b], Yan-Hong Wang [b], Yan-Hui Huang [a], Xia Liu [a], Xiao-Xia Wang [a], Wan Li [a], Xin Zhao [a], Li-Ping Wang [a], Shuai Yan [a], Di Wu [a], Yong-Jie Wu [a,*]

[a] Key Laboratory of Preclinical Study for New Drugs of Gansu Province, Department of Pharmacology, School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, Gansu, PR China
[b] Gansu Provincial People's Hospital, Lanzhou 730000, Gansu, PR China

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ABSTRACT

In this study, we investigated the effects and the protective mechanism of ginsenoside Rd (GRd) which has been identified as one of the effective compounds from ginseng on relapsing colitis model induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) in rats. After inducing relapsing colitis in experimental rats on two occasions by intracolonic injection of TNBS, GRd (10, 20 and 40 mg/kg) was administered to experimental colitis rats for 7 days. The inflammatory degree was assessed by macroscopic score, histology and myeloperoxidase (MPO) activity. The levels of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 were determined by ELISA. Mitogen-activated protein kinase (MAPK) phosphorylation was analyzed by western blotting method. The results showed that GRd markedly attenuates the inflammatory response to TNBS-induced relapsing colitis, as evidenced by improved signs, increased body weight, decreased colonic weight/length ratio, reduced colonic macroscopic and microscopic damage scores, inhibited the activity of MPO, lowered proinflammatory cytokine levels and suppressed phosphorylation of p38 and JNK. The possible mechanism of protection on experimental colitis after GRd administration was that it could reduce the accumulation of leukocytes and down-regulate multiple proinflammatory cytokines through modulation of JNK and p38 activation.

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1. Introduction

Inflammatory bowel disease (IBD) is characterized as a chronic idiopathic inflammation of the intestine. It is an umbrella term for different diseases of which ulcerative colitis and Crohn's disease (CD) are the two most common entities [1]. At present, medical treatment of IBD relies mainly on glucocorticoids, 5-aminosalicylic acid, and immunosuppressive agents. The limitations in both efficacy and safety encountered continue to drive the search for better therapeutic options [2]. IBD presents a challenging target for drug delivery, particularly by the oral route, as contrary to most therapeutic regimens, minimal systemic absorption and maximal intestinal wall drug levels are desired [3].

Ginseng is a perennial herb of the Araliaceae family, species in the genus *Panax*, and a highly valued medicinal plant in the Far East that has gained popularity in the West during the past decade [4,5]. Ginseng is reported to have a wide range of therapeutic and pharmacological uses [6]. Researchers are now focused on using purified individual ginsenoside to reveal the mechanism of functions of ginseng instead of using whole ginseng root [7,8]. Ginsenosides appear to be responsible for most of the activities of ginseng including vasorelaxation, antioxidation, anti-inflammation and anticancer. Ginsenoside Rd (GRd) has been identified as one of the effective compounds responsible for the pharmaceutical actions of ginseng [9]. GRd is a putative antioxidant in that it targets many of the key players involved in inflammation [10–12]. Wu et al. [10] suggested that GRd exerted its anti-inflammatory effects by inhibiting proinflammatory cytokines (such as tumor necrosis factor-α (TNF-α)) production and interfering with mitogen-activated protein kinase (MAPK) signaling pathways. Tamura et al. [13] reported that GRd may prevent and rescue rat intestinal epithelial cells from irradiation-induced apoptosis. Since IBD is a chronic inflammatory disease, we supposed that GRd can be used to treat colitis.

Generally, ginsenosides are very poorly absorbed following oral administration in vivo [14]. Wang et al. [15] reported the absolute bioavailability of GRd in dogs was 0.26%. Li et al. [16] reported the absolute bioavailability of GRd in rats was 0.34%. Our laboratory's results revealed that most of the GRd was found in the colon after oral administration (to be published). One of the reasons of the poor bioavailability of GRd is that it may be metabolized by colonic microflora [17]. This pharmacokinetic characteristic suggests that GRd affects the

* Corresponding author. Tel.: +86 931 13893445805; fax: +86 931 8915023.
E-mail address: wuyj@lzu.edu.cn (Y.-J. Wu).

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colonic mucosa directly, so that it has a natural colon-targeting feature that may be of therapeutic interest in colitis.

Based upon anti-inflammatory and pharmacokinetic properties of GRd, we examined the protective role and mechanism of GRd against 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced relapsing colitis in rats.

2. Materials and methods

2.1. Drugs and reagents

GRd was obtained from Guangdong Taihe Biological Pharmaceutical Co. Ltd. (Gongdong, China). Sulfasalazine (SASP) was supplied by Sine Pharmaceutical Co. Ltd. (Shanghai, China). TNBS was obtained from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Myeloperoxidase (MPO) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Rat TNF-α, interleukin-1β (IL-1β), and IL-6 platinum ELISA kits were purchased from Bender (Bender MedSystems, CA, USA). RIPA Lysis Buffer was purchased from Beyotime Institute Biotechnology (Jiangsu, China). The antibodies against extracellular signal regulated kinase (ERK), c-Jun NH_2-terminal kinase (JNK), p38, phospho-ERK, phospho-JNK, and phospho-p38 were purchased from Abcam Inc. (MA, USA). Goat anti-rabbit or Goat anti-mouse horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology, CA, USA) were used as secondary antibody. All other chemicals used were of analytical grade.

2.2. Drug and agent preparation

GRd (1, 2 and 4 mg) and SASP (50 mg) were each suspended in 1 mL of physiologic saline; 25 mg of TNBS was dissolved in 1 mL of 50% (v/v) ethanol.

2.3. Animal

A total of 60 male Wister rats 9–10 weeks of age, weighing 100–200 g, were provided from the Animal Experiment Center of Gansu College of Traditional Chinese Medicine. The rats were housed in standard cages (temperature 22–25 °C, relative humidity 70–75%, 12 hour light/dark cycle) and were fed with standard laboratory food. Animal experiments were conducted according to the principles of acceptable laboratory animal care. All experiments were approved by the ethics committee for laboratory animal care and use of Lanzhou University.

2.4. Induction of relapsing colitis in rats

Relapsing colitis was induced with TNBS enema by the modified methods of Li et al. [18]. The rats were anesthetized with sodium pentobarbital (30 mg/kg) by intraperitoneal route after 48 hour fast. An obtuse cannula was inserted into the anus of the rats, and the tip was advanced approximately 8 cm. TNBS (100 mg/kg) was instilled into the colon through the cannula. Following the instillation of the TNBS, the rats were kept in a head-down position for 30 min to prevent leakage of the intracolonic instillation. To establish a relapsing colitis model, rats were instilled a second time with TNBS at a dose of 37.5 mg/kg into the colon on the fourteenth day after the first induction of relapsing colitis [19]. The remainder of the process was the same as in the first induction phase. The rats from the normal group were instilled with physiological saline at 4 and 1.5 ml/kg in the first and second induction phases, respectively.

2.5. Experimental design

The rats were randomly divided into six groups ($n=10$ per group). Twenty-four hours after the second induction of relapsing colitis, treatment began and was continued for 7 days. GRd (10, 20 and 40 mg/kg) was orally administered once daily in a volume of 10 mL/kg body weight respectively. The positive control group received SASP in a dose of 500 mg/kg once daily. Normal and model groups were treated with physiological saline in a volume of 10 mL/kg body weight, respectively. The rats were checked daily for behavior, body weight, and stool consistency. On the eighth day after the second induction of relapsing colitis, rats were sacrificed using an overdose of anesthetic, and laparatomy was performed. Colonic segments were excised, freed of adherent adipose tissue, rinsed with saline to remove fecal residue, then blotted, weighted, and measured.

2.6. Assessment of macroscopic damage

Macroscopic damage was assessed by the scoring system of Gálvez et al. [20] which relates to the area of inflammation and the presence or absence of ulcers. The criteria for assessing macroscopic damage and the numerical rating score were as follows: 0, no damage; 1, hyperemia, no ulcers; 2, linear ulcer with no significant inflammation; 3, linear ulcer with inflammation at one site; 4, two or more sites of ulceration or inflammation and ulceration or inflammation extending <1 cm; and 5, two or more major sites of ulceration or inflammation extending >1 cm along the length of the colon.

2.7. Assessment of microscopic damage

Representative colon specimens were taken from an area of the treated colon segment, and were fixed in 4% paraformaldehyde. The formalin-fixed colon tissues were embedded in paraffin wax, and sections 5 μm in length were stained with hematoxylin–eosin for pathomorphological examination. The criteria for assessing microscopic damage were as follows: 0, normal colonic tissue; 1, inflammation or focal ulceration limited to the mucosa; 2, focal or extensive ulceration and inflammation limited to the mucosa and the submucosa; 3, focal or extensive ulceration and inflammation limited with involvement of the muscularis propria; 4, focal or extensive ulceration and inflammation limited with involvement of the serosa; and 5, extensive ulceration and transmural inflammation with involvement of the serosa [20].

2.8. Determination of colon MPO activity

One gram of colon tissue was weighted and homogenized in 9 mL of cold physiological saline. The MPO activity was determined with the O-dianisidine method [21] using a MPO detection kit. The MPO activity was measured spectrophotometrically by absorbance at 460 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide per minute at 25 °C and was expressed in units per gram weight of wet tissue (U/g tissue).

2.9. ELISA determination of TNF-α, IL-1β, and IL-6 levels in colon tissues

One gram of colon tissue was weighted and homogenized in 9 mL of cold physiological saline. The homogenate was centrifuged at $12{,}000\times g$ for 10 min; the supernatant was transferred into several new tubes, and stored at −80 °C until analysis. The concentration of TNF-α, IL-1β, and IL-6 in colon tissues was quantified by an enzyme-linked immunoassay kit, respectively. The results were expressed as picograms per gram of wet tissue (pg/g tissue).

2.10. Western blotting analyses for ERKs, p38, and JNK phosphorylation

Colon tissue was homogenized in ice-cold RIPA lysis buffer (1% Triton X-100, 0.1% SDS, 0.5% deoxycholate, 1 mM EDTA, 20 mM Tris (pH 7.4), 150 mM NaCl, 10 mM NaF, 1 mM Na_3VO_4, 0.1 mM phenylmethylsulfonyl fluoride). After homogenization, tissue lysates were

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