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Proanthocyanidins from grape seeds modulates the nuclear factor-kappa B signal transduction pathways in rats with TNBS-induced recurrent ulcerative colitis

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ABSTRACT

The aim of this study was to elucidate the molecular mechanisms involved in the therapeutic effects of proanthocyanidins from grape seeds (GSPE) on recurrent ulcerative colitis (UC) in rats. GSPE in doses of 100, 200, and 400 mg/kg were intragastrically administered per day for 7 days after recurrent colitis was twice-induced by TNBS. The levels of GSH, as well as the activity of GSH-Px and SOD in colon tissues were measured by biochemical methods. The expression levels of tumor necrosis factor-α (TNF-α) and the nuclear translocation levels of nuclear factor-kappa B (NF-κB) in the colon tissues were measured by enzyme-linked immunosorbent assay methods. Western blotting analysis was used to determine the protein expression levels of inhibitory kappa B-alpha (IκBα), inhibitor kappa B kinase (IKKα/β), phosphorylated IκBα and phosphorylated IKKα/β. GSPE treatment was associated with a remarkable increased the activity of GSH-Px and SOD with GSH levels in TNBS-induced recurrent colitis rats as compared to the model group. GSPE also significantly reduced the expression levels of TNF-α, p-IKKα/β, p-IκBα and the translocation of NF-κB in the colon mucosa. GSPE exerted a protective effect on recurrent colitis in rats by modifying the inflammatory response and promoting damaged tissue repair to improve colonic oxidative stress. Moreover, GSPE inhibited the TNBS-induced inflammatory of recurrent colitis though blocking NF-κB signaling pathways.

1. Introduction

Ulcerative colitis (UC) is a recurrent, idiopathic inflammatory disorder involving the mucosa and sub-mucosa of the colon. Although the etiology and pathophysiology remain unclear, immune dysfunction plays a crucial role in the development of UC [1]. Inflammatory mediators, including reactive oxygen species (ROS) and cytokines, contribute to the inflammatory cascade in modulating the immune system of UC [2–4]. Proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β) in the colonic mucosa significantly increased and antiinflammatory cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10) significantly decreased in UC [5]. Of the various kinds of inflammatory mediators, TNF-α, which is induced, synthesized and secreted from macrophages, lymphocytes, and polymorphonuclear neutrophils, is regarded as the most prominent "first-line" cytokines [6,7]. TNF-α can induce the production of reactive oxygen species, and it can activate oxidative stress-responsive genes which amplify and prolong inflammation [8].

The nuclear transcription factor NF-κB has a crucial role in the pathogenesis of several human disorders, particularly those with an inflammatory component. The signaling pathways of NF-κB were activated dependent on IKK. In the canonical pathway, the stimulation of receptors such as the TNF receptor, Toll-like receptors (TLRs) or the T-cell receptor (TCR) activates a multisubunit IKK (IKKα, IKKβ and IKKγ), which catalyzes the phosphorylation of IκBα. This phosphorylation is essential for signaling the subsequent ubiquitination and proteolysis of IκBα, leaving NF-κB free to translocate to the nucleus and promote gene transcription. Once activated, NF-κB translocated to the nucleus from the cytoplasm, then activated the consensus sequence related gene, including TNF-α, IL-6, IL-2, IL-8, ICAM-1, and so forth, involved in immune and inflammatory responses [9,10]. Increasing evidence reveals that the inhibition of NF-κB activity may lead to alleviating the severity of inflammatory diseases [11]. Therefore, understanding the molecular mechanisms involved in this pathway is an essential step towards countering the damaging effects of pro-inflammatory mediators in UC.

A number of animal models of acute and chronic colon inflammation have been developed to mimic human UC. We investigated the TNBS-induced colitis model in rats because this model is well known to have a caustic tissue injury and an immunology component which can be reactivated into relapse by systemic antigen treatment. The recurrent colitis model, which was twice induced by TNBS [12], as a

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good experimental model to mimic a classic course of human chronic-recurrent ulcerative colitis, as well as afford an opportunity to study the pathogenesis of colonic inflammatory disease and may be used to evaluate new treatments potentially applicable to UC.

Proanthocyanidins from grape seeds (GSPE), are naturally occurring polyphenolic bioflavonoids, have a strong anti-inflammatory activity [13,14]. Pharmacokinetic studies have confirmed that there is little or no absorption of GSPE in the stomach and small intestine when taken orally and that degradation occurs mainly in the colon [15]. This metabolic characteristic suggests that GSPE affects the colonic mucosa directly, so that it was named a natural colon-targeting feature that may be of therapeutic interest in UC. Our previous study has demonstrated that GSPE at one dose of 200 mg/kg exerts a beneficial antiinflammatory effect in the recurrent phase of TNBS-induced colitis in rats [12]. However, the pivotal elements of its inhibitory action on the inflammation remain unclear. To elucidate the molecular mechanisms involved in the therapeutic effects of GSPE on regulating the inflammatory response of TNBS induced recurrent colitis in rats, we examined the influence of GSPE on the NF-κB signaling pathways. Furthermore, we determined the dose-effect relationship of GSPE that exerted the antiinflammatory effect.

2. Materials and methods

2.1. Animals

Male Wistar rats, 180–200 g and 9–10 weeks of age were obtained from the Experiment Animal Center of Gansu College of Traditional Chinese Medicine (Consent No. SCXK (G) 2004–0006). The rats were housed in plastic cages in a room and kept under standardized conditions at a temperature of 22–24 °C, and 20% humidity with a 12 h light/dark cycle, and they had free access to tap water and food throughout the study. They were allowed to acclimatize for 1 week before the experiments were started. Animal experiments were conducted under principles in good laboratory animal care, and approved by ethical committee for Laboratory Animals Care and Use of Lanzhou University.

2.2. Drugs and reagents

GSPE was obtained from Tianjin Jianfeng Natural Products Company (quality and specification: proanthocya-nidins >95%, imeric >1.8%, oligomers >60%; Tianjin, China). Sulfasalazine (SASP) was obtained from Shanghai Sine Jiahua Pharmaceutical (Shanghai, China). 5% (*w*/*v*) TNBS was obtained from Sigma-Aldrich, Inc. (St Louis, USA). Kits of superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GSH-Px), and Coomassie brilliant blue protein assay were all purchased from the agent of Nanjing Jiancheng bioengineering Institute (Jiangsu, China). ELISA kits of rat nuclear factor-kappa B (NF-κB) were purchased from Uscn life Science Inc. (Wuhan, China). Rat tumor necrosis factor-α (TNF-α) platinum ELISA kit was purchased from Bender (Bender MedSystems, CA, USA). The antibodies against IKKα/β, p-IKKα/β, IκBα and p-IκBα were purchased from Abcam Inc. (USA). Goat anti-rabbit or Goat anti-mouse horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology, CA, USA) was used as secondary antibody. All other chemicals used were of analytical grade.

2.3. Induction of experiment colitis in rats and treatment protocols

All rats were randomly divided into 6 groups: normal group (n = 10), TNBS-induced recurrent UC group (model group, n = 10), SASP group (n = 10) as a positive control, and the 3 GSPE treatment groups (each n = 10), which consisted of low dose (GSPE-L, 100 mg/kg), medium dose (GSPE-M, 200 mg/kg), and high dose (GSPE-H, 400 mg/kg). The dosage of SASP or GSPE was 10 mL/kg, once daily, for a total of 7 days. These dosages were chosen on the basis of a previous study, as they were the most active in preventing acetic acid or TNBS induced acute colitis in rats [16,17].

2.3.1. Induction of recurrent colitis in rats

Colitis was induced according to the procedure described by Li et al. [17] and Wang et al. [12]. Briefly, rats were fasted for 48 h, and then anesthetized with sodium pentobarbital (30 mg/kg i.p.). An obtuse cannula was inserted into the anus of the rats, and the tip was advanced approximately 8 cm. Rat from model group and the drug-treated groups were installed with TNBS at a dose of 80 mg/kg (20 mg of TNBS was dissolved in 1 mL of 50% ethanol (*v*/*v*), 4 mL/kg) through the cannula in the first induction phase. 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 simulate a classic course of human chronic-recurrent UC, the rats were second instilled with TNBS at doses of 30 mg/kg (20 mg of TNBS was dissolved in 1 mL of 50% ethanol (*v*/*v*), 1.5 mL/kg) into the colon on the sixteenth day after the first induction UC. The remainder of the process was the same as the first induction phase. Rats in the normal group received physiological saline, instead of TNBS solution.

2.3.2. Treatment

Low, medium, and high (10, 20, and 40 mg) doses of GSPE and 50 mg of SASP were each suspended in 1 mL of physiological saline. Twenty-four hours after second TNBS-induced colitis, treatment began and continued for 7 days. Rats in the SASP and GSPE groups were intragastrically given SASP or GSPE, respectively. The rats from the model group and the normal group were administrated intragastric physiological saline. During the study, rats were checked daily for behavioral changes, stool consistency, and body weight.

2.4. Assessment of colitis

All the rats were sacrificed after treatment with SASP or GSPE was continued for 7 days. The severity of colitis was evaluated by an independent observer who was blind to the treatment. For each animal, the entire colon was excised and cleaned of adherent adipose tissue (removing the cecum and appendix), rinsed with ice-cold saline, opened longitudinally and washed with cold physiological saline to remove fecal residues. The colon was examined visually immediately and damage was scored on a scale of 0–5 according to the criteria described by Galvez et al. [18]. Scoring of macroscopic colon damage in TNBS-induced colitis was: 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; 5, two or more major sites of ulceration or inflammation extending >1 cm along the length of the colon. The adhesions were presented between colon, small bowel and other organs (score 0–2) according to the criteria of Bobin-Dubigeon et al. [19]. Representative colon specimens were taken from a region of the inflamed site adjacent to the macroscopic damage of each rat, and were fixed in 4% buffered formaldehyde. The formalin-fixed colon tissues were embedded in paraffin, and sections 5 μm in length were stained with hematoxylin and eosin (H&E) for histological evaluation of colonic damage by light microscopy according to criteria described by Galvez et al. [18]. Scoring of microscopic colon damage in TNBS-induced colitis is 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.