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Parthenolide, an inhibitor of the nuclear factor-κB pathway, ameliorates dextran sulfate sodium-induced colitis in mice

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ABSTRACT

Background: Activation of nuclear factor-kappa B (NF-κB), which controls transcription of various pro-inflammatory cytokine genes, has been shown to play a critical role in the pathogenesis of ulcerative colitis (UC). Parthenolide, a sesquiterpene lactone compound isolated from extracts of the herb Feverfew (*Tanacetum parthenium*), has been demonstrated to be a potent inhibitor of NF-κB activation. This study was designed to investigate the effects of parthenolide on an experimental murine colitis model.
Materials and methods: Experimental colitis was induced by dextran sulfate sodium (DSS), and mice were divided into 3 groups: normal control, DSS + saline, and DSS + parthenolide. The disease activity index (DAI) and histological score were observed. The tumor necrosis factor (TNF)-α and interleukin (IL)-1β levels were measured by enzyme-linked immunosorbent assay. Phospho-IκBα, IκBα and phospho-NF-κB p65 expression were assessed by western blot analysis. Myeloperoxidase (MPO) activity was determined by using MPO assay kit.
Results: Administration of parthenolide significantly reduced the severity of DSS-induced colitis as assessed by DAI and histological score, and resulted in downregulation of MPO activity and phospho-NF-κB p65 expression by the blockade of phosphorylation and subsequent degradation of IκB protein, strikingly reduced the production of TNF-α and IL-1β.
Conclusion: Parthenolide exerts beneficial effects in experimental colitis and may therefore provide a useful therapeutic approach for the treatment of UC.

1. Introduction

Ulcerative colitis (UC) is a chronic and relapsing condition of the intestinal inflammation of unknown etiology and remains one of the most intractable gastrointestinal diseases, impairing quality of life and carrying a high risk of colorectal cancer in patients with UC [1]. Although much progress has been made in the management of the disease, definitive causal therapies for human UC until now are not available. In fact, 5-aminosalicyclic acid and its derivatives are still the drugs of choice for current medical treatment; corticosteroids, azathioprine, mercaptopurines and cyclosporine are used in more severe forms of the disease. However, the therapies not only show limited benefits but also have serious side effects [1,2]. Recently, a biological agent, anti-tumor necrosis factor alpha (anti-TNF-α) humanized antibody (infliximab), has been demonstrated to be effective in clinical application for UC. Unfortunately, this new drug has also been shown to have serious adverse reactions, such as increased risk of infection, hypersensitivity and anti-antibody reaction, and an unknown risk of mutagenesis, thus limiting its use [3,4]. Therefore, there is a pressing need for developing effective and specific therapeutic approaches for UC.

Although the exact etiologies of UC still remain elusive, considerable evidence gained over the past decade has supported that the over-expression of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-12, and tumor necrosis factor (TNF)-α plays an important role in pathogenesis of UC [5,6]. Indeed, studies from patients with UC have shown increased expression of certain proinflammatory cytokines such as IL-1, IL-6, and TNF-α in inflamed mucosal tissues [5–7]. And further studies have also shown that all these proinflammatory cytokine genes include nuclear factor-κB (NF-κB) binding sites and are transcriptionally regulated by the factor [8]. The NF-κB family comprises five members (relA [p65], relB, c-Rel, p105/50, and p100/52) associated as homo or heterodimers. In unstimulated cells, the majority of NF-κB dimers are inactivated and retained in the cytoplasm by association with small inhibitory subunit called IκB. Upon stimuli, IκB proteins are phosphorylated, ubiquitinated, and degraded, allowing NF-κB to translocate to the nucleus where it can bind specific DNA sequences located in the promoter regions of target genes and activate gene transcription, indicating its pivotal role in regulation of inflammation by controlling the transcription of

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inflammatory cytokine genes [9,10]. In fact, the increased NF-κB expression in mucosal macrophages of patients with UC, accompanied by an increased capacity of these cells to produce and secrete TNF-α, IL-1 and IL-6 has been demonstrated [11,12]. The finding reflects the central role of NF-κB in controlling secretion of proinflammatory cytokines, which are directly involved in the mucosal tissue damage occurring in UC, and indicates that the activation of NF-κB and its subsequent increase of proinflammatory cytokine expression play a critical role in pathogenesis of UC. Consequently, the effective inhibition of the pathway of NF-κB-regulated cytokine expression may be beneficial for the treatment of UC. Indeed, previous studies had shown that the administration of antisense oligodeoxynucleotides (ODNs) against NF-κB or double-stranded decoy ODNs against NF-κB effectively reduced production of proinflammatory cytokines and abrogated established colonic inflammation in murine models of colitis [13–15]. Parthenolide, a sesquiterpene lactone compound, is isolated from extracts of the herb Feverfew (*Tanacetum parthenium*), which has been used widely as folk remedies for inflammatory conditions such as migraine, arthritis, asthma, and so on [16–22]. Several studies have demonstrated that parthenolide is a potent inhibitor of NF-κB activation and can inhibit the expression of pro-inflammatory cytokines in cultured cells and experimental models [16–22], implying that parthenolide may be developed to a potential strategy in treatment for UC. Unfortunately, to date, there has been no information on whether parthenolide is therapeutic for UC. Therefore, we hypothesized that parthenolide could exert its anti-inflammatory effect on UC by inhibiting the activation of NF-κB signaling pathway. Here, we investigated the effect of parthenolide on a murine model of dextran sulfate sodium (DSS)-induced colitis, which resembles human UC, in order to provide experimental evidence that parthenolide serves as a possible treatment for patients with UC.

2. Materials and methods

2.1. Animals

Eight-week-old male BALB/c mice weighing about 18–23 g were obtained from the Experimental Animal Center of Sichuan University, Chengdu, China and housed in cages at room temperature (25 °C) with alternating 12:12-h light–dark cycles. Standard mouse chow pellets and water were supplied ad libitum. This study was approved by the Animal Ethics Committee of West China Hospital, Sichuan University.

2.2. Induction of colitis and administration of parthenolide

Colitis was induced by giving 5% DSS (molecular weight 36–50 kDa, MP Biomedicals, Aurora, OH, USA) orally in drinking water for 7 days ad libitum. Mice were randomized into three groups. The first group, designated as normal control group (n = 8), received tap water for 7 days. The second group, designated as DSS control group (n = 8), received 5% DSS for 7 days and 0.5 ml of saline given intracolonically on day 7 and continued for an additional 5 days. The third group, designated as parthenolide group (n = 8), received 5% DSS for 7 days and 10 mg/kg of parthenolide in 0.5 ml of saline solution intracolonically on day 7 and continued for an additional 5 days. Intracolonic administration was done by slow infusion through a polyethylene catheter inserted 4 cm into the anus. The dosage of parthenolide (Biopurify Phytochemicals Ltd, Chengdu, PR China; purity > 98%) was chosen on the basis of a preliminary experiment carried out, in which various doses of parthenolide ranging from 5, 10 and 15 mg/kg were administered intracolonically. 10 mg per kilogram of parthenolide, which had been shown to result in effective inhibition of NF-κB activity (see Fig. 1) and demonstrated previously to be protective in other animal model [23], was used in subsequent experiments. Mice were killed under anesthesia on day 13.

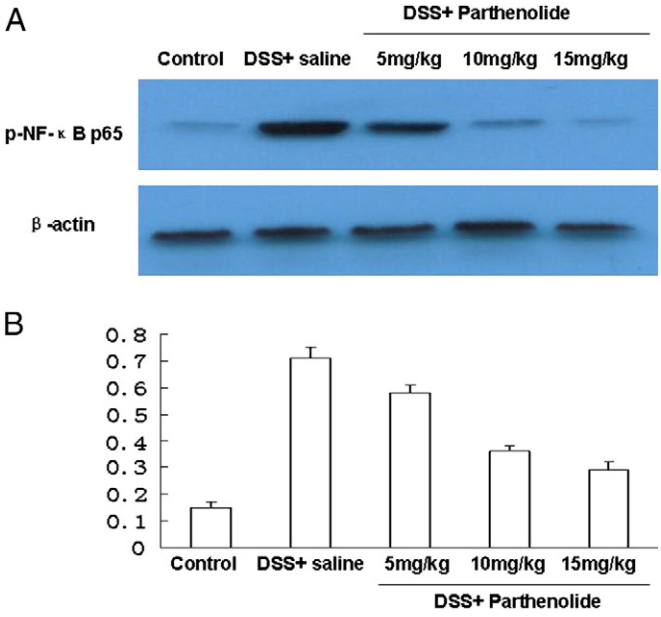

Fig. 1. A). Western blot results display protein expression of phospho-NF-κB p65 after treatment with parthenolide in various doses. B). Densitometric analysis of the expression of phospho-NF-κB p65. Parthenolide at low dose (5 mg/kg) slightly reduced the expression of phospho-NF-κB p65, whereas treatment with higher doses (10, 15 mg/kg) was more effective (each group, n = 3).

2.3. Evaluation of colitis

A disease activity index (DAI) was determined by scoring changes in body weight, stool Hemoccult positivity, or gross bleeding and stool consistency in accordance with the method described by Murthy et al. [24]. After the mice were killed under anesthesia, their colons were immediately removed and fixed in 10% buffered formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Histological score of H&E-stained specimens of the colon was determined by two pathologists in a blinded fashion according to the method reported by ten Hove et al. [25]. The mean score in each section was calculated.

2.4. Western blot analysis

To determine the effects of parthenolide on phospho-IκBα, IκBα and phospho-NF-κB p65 in colonic tissue, the extracts from colonic tissue (whole colonic layer) were prepared using commercial extract kit (Active Motif, Carlsbad, CA, USA) and were analyzed by western blot according to standard protocols. In brief, the extracts that contained 40 μg proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane (Invitrogen, Carlsbad, CA, USA). After being blocked with 5% nonfat dry milk, the membrane was incubated at 4 °C overnight with the anti-phospho-NF-κB p65 antibody (1:1000), anti-phospho-IκBα (1:1000), anti-IκBα (1:1000) and anti-β-actin antibody (1:500), respectively, and then with a horseradish peroxidase-conjugated secondary antibody (1:5000) for 1 h. All of the antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The immune complexes were visualized with the ECL system (Amersham Pharmacia Biotech Inc, Arlington, USA), and the bands were measured by a Fluorchem imaging system (Alpha Innotech Corp, San Leandro, CA, USA) for quantitative densitometric analysis.