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Administration of geniposide ameliorates dextran sulfate sodium-induced colitis in mice via inhibition of inflammation and mucosal damage

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ABSTRACT

Ulcerative colitis (UC), an idiopathic inflammatory bowel disease, not only affects millions of patients worldwide, but also increases the risk of colon cancer. Geniposide is an iridoid glycoside and has many biological activities such as anti-inflammatory and antioxidant. However, its protective efficacy and mechanism of action against UC are still unclear. In this study, we aimed to investigate the protective effects and mechanisms of geniposide on dextran sulfate sodium (DSS)-induced experimental colitis in mice. The results revealed that geniposide alleviated body weight loss, disease activity index, colon length shortening and colonic pathological damage induced by DSS. Geniposide significantly suppressed pro-inflammatory cytokines by regulating NF-κB and PPARγ pathways *in vivo* and *in vitro*. Furthermore, geniposide also significantly regulated the expressions of ZO-1 and occludin in DSS-induced experimental colitis in mice and lipopolysaccharide (LPS)-triggered inflammation in Caco-2 cells. These findings indicated that geniposide may be a new natural chemopreventive agent to combat UC.

1. Introduction

Ulcerative colitis (UC) is a nonspecific, chronic and relapsing inflammation of the gastrointestinal. Abdominal pain, diarrhea, purulent stools and relapses are the main clinical characteristics of UC. It not only affects millions of patients worldwide, but also increases the risk of colon cancer [1,2]. Although the precise etiology of UC remains uncertain, the intestinal mucosa of UC patients is reported to be characterized by an inappropriate and sustained activation of the mucosal immune system which will damage the intestinal epithelial barrier by the abnormal activity of some pro-inflammatory signals [3]. Peroxisome proliferator-activated receptor γ (PPARγ) is an important anti-inflammatory mediator during colonic inflammation [4]. Activation of PPARγ suppresses the activation of the nuclear factor-κB (NF-κB) signaling pathway, inhibits the expression of inflammatory cytokines and neutrophil infiltration in intestinal mucosa [5]. In addition, the epithelial barrier is beneficial to the intestinal function of improving healthy gastrointestinal tract [6]. The disturbance in the colonic tight junction (TJ) has been reported to have a significant impact on the pathogenesis of UC patients [7].

Currently, most therapeutic drugs for UC mainly includes glucocorticosteroids, immunosuppressive agents and anti-TNF-a monoclonal antibody [8]. Unfortunately, most of these agents are effective for only temporary symptomatic and are expensive, particularly for long-term therapy [9]. Natural products derived from medicinal plants or herbs have been developed as an important complementary treatment for UC [10]. Geniposide is one of the main bioactive components of gardenia fruit. It has been showed that geniposide could inhibit cytokines production by regulating NF-κB pathway in human umbilical vein endothelial cells [11]. Our previous study has also reported that geniposide inhibits the inflammatory response in the primary mouse macrophages and mouse models of acute lung injury [12]. However, there is little information about the effects of geniposide on UC. Thus, this study was designed to determine the protective effect of geniposide on DSS-induced mice colitis and explore the underlying mechanisms.

2. Materials and methods

2.1. Chemicals

Geniposide was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Jilin, China) with > 98% purity. Antibodies against p65, p-p65, IκB and p-IκB were provided by Cell Signaling Technology Inc. (Beverly, MA, USA).

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Table 1
Oligonucleotide primers used for qRT-PCR.

Name	Primer sequence
TNF-α	Sense: 5′-GCCTCCCTCTCATCAGTTCTA-3′ Anti-sense: 5′-GGCAGCCTTGTCCCTTG-3′
IL-6	Sense: 5′-AGTTGTGCAATGGCAATTCTGA-3′ Anti-sense: 5′-AGGACTCTGGCTTTGTCTTTCT-3′
IL-1β	Sense: 5′-ACCTGTGTCTTTCCCGTGG-3′ Anti-sense: 5′- TCATCTCGGAGCCTGTAGTG-3′
β-Actin	Sense: 5′-CTACCGTCGTGACTTCGC-3′ Anti-sense: 5′-GGGTGACATCTCCCTGTT-3′

Primary antibodies that were raised against zonula occludens-1 (ZO-1) and occludin were obtained from Santa Cruz (Santa Cruz, USA). Mouse mAb PPARγ were purchased from GeneTex. β-Actin and horseradish peroxidase conjugated goat anti-rabbit and goat anti-mouse antibodies were purchased from Tianjin Sungene Biotech Co., Ltd. (Tianjin, China). The myeloperoxidase (MPO) determination kit was purchased from the Jiancheng Bioengineering Institute of Nanjing (Nanjing, China). Mouse tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Biolegend (San Diego, CA, USA). All other chemicals were of reagent grade.

2.2. Cell culture and viability assay

Human intestinal epithelial cells (Caco-2 cells) were cultured with 15% FBS/DMEM-F12 supplied with 1 mM sodium pyruvate and 50 U/ml penicillin–streptomycin. Caco-2 cells were maintained at 37 °C in a humidified 5% CO_2 incubator. Cells were pretreated with or without geniposide (50, 100 and 200 μg/ml) for 1 h. After that, cells were treated with LPS (1 μg/ml). After 18 h of LPS stimulation, MTT (20 μl of 5 mg/ml) was added to each well for 4 h. The supernatant was removed and dimethyl sulfoxide (150 μl per well) was added. The optical density was tested at 570 nm using a microplate reader.

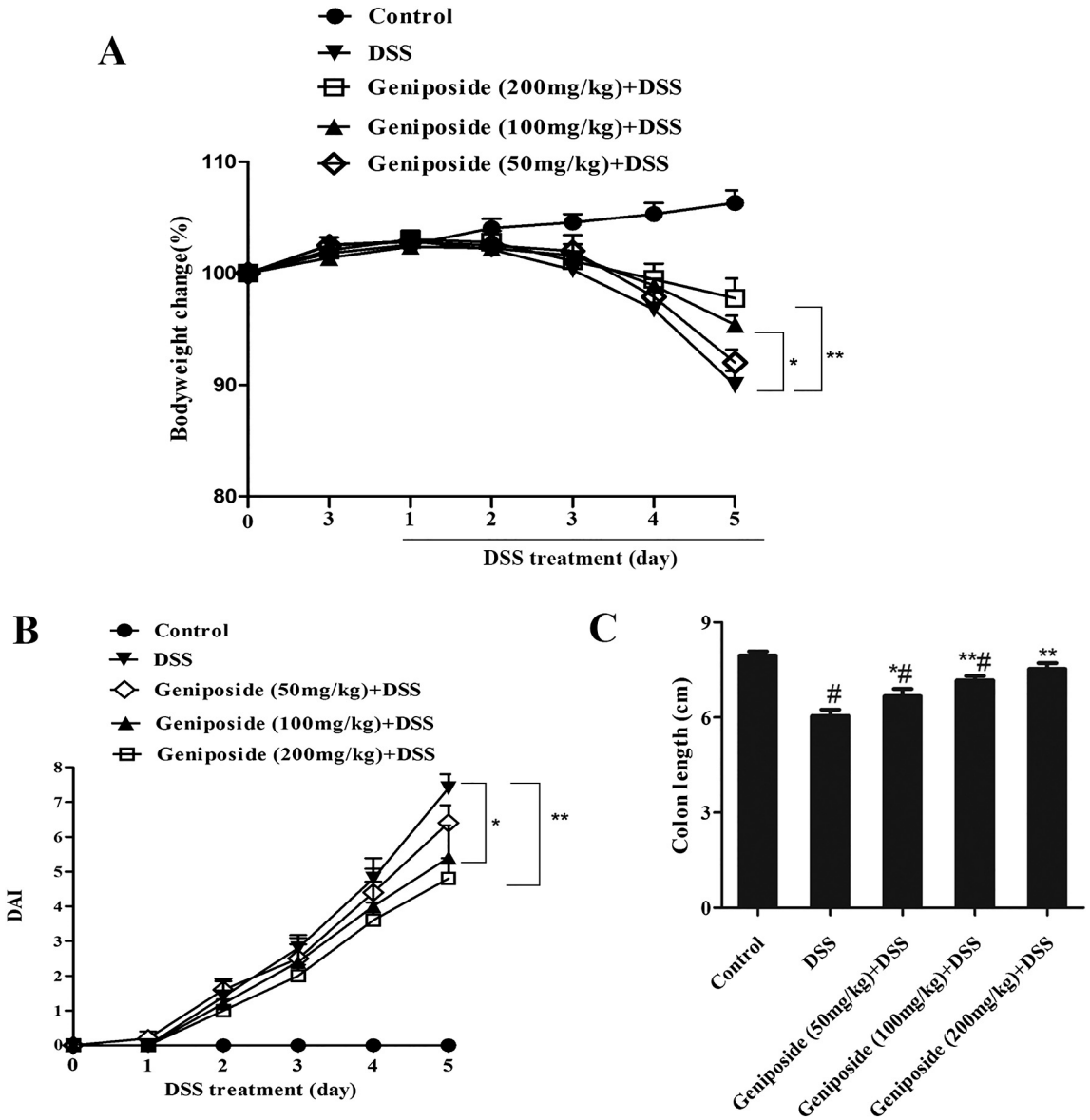

Fig. 1. Geniposide attenuated DSS-induced colitis in mice. (A) Body weight change of each group. (B) Disease activity index (DAI). (C) The lengths of colons from each group of mice were measured. Data were presented as the means ± SD ($n = 6$ per group). Body weight and DAI differences were analyzed using unpaired two-tailed Student's *t*-test. Graph bars differences in the lengths of colons based on one-way ANOVA followed by Tukey's multiple-comparison test. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus the DSS-treated group; $^{\#}p < 0.05$ compared with control group.