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Original Article

Siegesbeckia pubescens ameliorates experimental ulcerative colitis in rats by modulating PPARγ

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Abstract: *Siegesbeckia pubescens* is a traditional Chinese medicine widely used for the treatment of inflammatory and autoimmune diseases in clinic. In order to evaluate its therapeutic effect against ulcerative colitis (UC) and elucidate its possible mechanisms, in this study, the effect of aqueous extract of *S. pubescens* (SPA) on 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced experimental UC in rats was determined. The results showed that oral administration of SPA significantly ameliorated colonic edema, hyperemia, inflammation and anabrosis in TNBS-induced experimental UC in rats. In addition, SPA inhibited MPO and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IFN-γ) production, and up-regulated PPARγ expression in colon. These results indicated that SPA has therapeutic effects against TNBS-induced experimental UC in rats, and its underlying mechanisms involve the modulation of PPARγ-mediated production of inflammatory mediators. These findings highlight the potential of this traditional Chinese medicine as an agent for use in the treatment of UC.

Keywords: Siegesbeckia pubescens, ulcerative colitis, TNBS, PPARγ

Introduction

Ulcerative colitis (UC) is a chronic idiopathic inflammatory bowel disease characterized by continuous mucosal inflammation that starts in the rectum and extends proximally. Its typical symptoms include bloody diarrhea, abdominal pain and mucus stool. Unfortunately, the etiology and pathogenesis of UC are still poorly understood, and the available therapies, including conventional anti-inflammatory agents (such as corticosteroids and 5-aminosalicylic acid), immunosuppressants and biologicals, are not completely effective in eliminating the disease [1]. Accordingly, the incidence and prevalence of UC in most of developed and developing countries has increased [2]. Therefore, it is necessary to develop novel therapeutic options for this global emerging disease.

Siegesbeckia pubescens Makino is a well-known traditional Chinese medicine clinically used in China for the treatment of inflammatory and autoimmune diseases, such as rheumatic arthritis and osteoarthritis [3]. It has also been reported that *S. pubescens* ameliorates acute enteritis in clinic [4]. However, although the anti-inflammatory activity of *S. pubescens* have been verified in animal models, the pharmacological mechanism of this action is not yet clearly defined, and there has been no report regarding its therapeutic effect on UC.

In this study, to evaluate the therapeutic effect of *S. pubescens* on UC, we examined the effect of aqueous extract of *S. pubescens* (SPA) on 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced experimental UC in rats. Furthermore, the mechanisms underlying the anti-inflammatory effect were discussed.

Materials and methods

Chemicals and reagents

TNBS were purchased from Sigma Chemical Co., St. Louis, USA. As the positive control, sul-

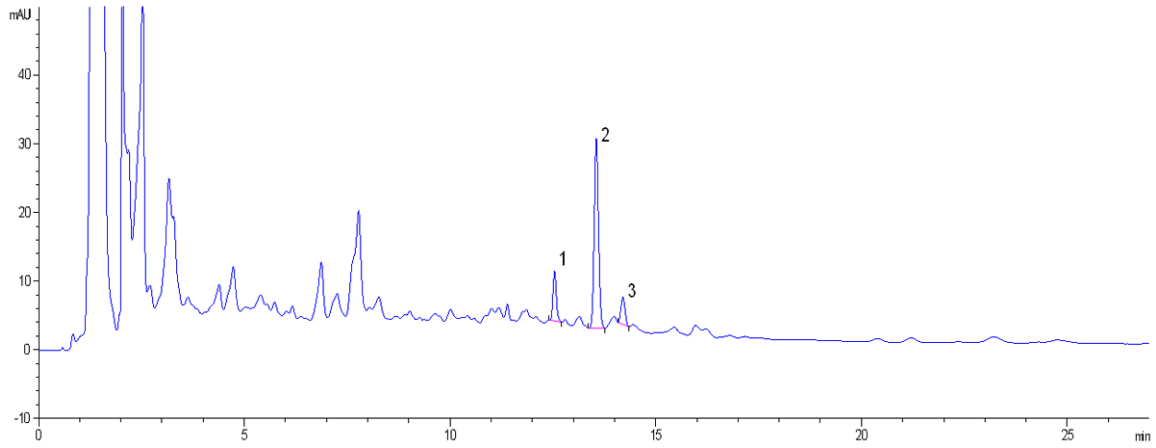

Figure 1. The HPLC Chromatogram of SPA. 1: Pubeside D; 2: Kirenol; 3: Darutoside.

fasalazine (SASP) was supplied by Shanghai Zhongxi Sunve Pharmaceutical Company Limited, Shanghai, China. Occult blood (OB) reagent (aminopyrine semi-quantitative test) was purchased from Zhuhai Baso Diagnostics Inc, Guangdong, China. Myeloperoxidase (MPO) kit was purchased from Nanjing Jiancheng Biological Engineering Institute, Nanjing, China. BCA protein concentration determination kit was purchased from Beyotime Institute of Biotechnology, Jiangsu, China. ELISA kits for TNF-α, IL-1β, IL-6, and IFN-γ were purchased from Wuhan boster biological engineering Co., Ltd., Wuhan, China. Trizol was purchased from Invitrogen, Carlsbad, CA, USA. PCR primers and other PCR reagents were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China. PPARγ and GAPDH rabbit polyclonal antibody were purchased from Santa Cruz Biotechnology Co., Ltd., Dallas, USA. IRDye 680LT secondary antibodies were purchased from Li-cor biosciences Inc., USA. All Other chemicals were of the highest purity and analytical grade.

Preparation of SPA

The aerial parts of *S. pubescens* Makino were obtained from East China Pharmaceutical Group Limited Company and identified by Prof. Xiaoyu Li (Zhejiang Academy of Medical Sciences, Hangzhou, China). A voucher specimen (No. 20121024) was deposited in Laboratory of Natural Products, Zhejiang Academy of Medical Sciences, Hangzhou, China. The plant material (1 kg) was extracted three times with water under boiling. The aqueous extract was filtered and evaporated on a rotary evaporator under reduced pressure to small volume and then lyophilized to yield a brown solid (named SPA, 170.0 g), which was then stored at 4°C until required.

To guarantee the quality of SPA, the amounts of presumed anti-inflammatory active constituents in SPA were determined. The content of total flavonoids was detected by aluminium colourimetric method and expressed as rutin equivalents (mg RE/g sample) [5]. Three diterpenoids (Kirenol, Pubeside D and Darutoside) in SPA were analyzed by HPLC. In brief, SPA (163.67 mg) was dissolved in methanol and diluted to 25 mL. 20 mL of the sample solution was injected to an Agilent 1260 HPLC apparatus (Agilent, USA) with a ZORBAX SB C18 (4.6 × 150 mm, 5 mm) column (column temperature 4°C) and acetonitrile/water (0-10 min: acetonitrile from 10% to 30%; 10-20 min: acetonitrile 30%) as mobile phase. The flow rate was 1.0 ml/min and the detection wavelength at 220 nm. The amounts of total flavonoids in SPA were 22.96 ± 0.05 mg RE/g. The contents of Kirenol, Pubeside D and Darutoside in SPA were 4.50 ± 0.10 mg/g, 0.80 ± 0.02 mg/g and 0.82 ± 0.02 mg/g, respectively (**Figure 1**).

Experimental animals

Sprague-Dawley rats (male, 180-220 g) were purchased from Zhejiang Experimental Animal Center (Hangzhou, China) and acclimatized for a week before use. Rodent laboratory chow and tap water were provided ad libitum, and maintained under controlled conditions: tempera-

ture 24 ± 1°C, humidity 50 ± 10%, 12-h light/12-h dark cycle. All the procedures were in strict accordance with the P. R. China legislation on the use and care of laboratory animals, and with the guidelines established by the Experimental Animals Center of Zhejiang Province, and were approved by the Animal Care and Use Committee of Zhejiang Academy of Medical Sciences, China.

TNBS-induced experimental UC in rats and drug administration

Experimental UC in rats was induced by TNBS according to the methodology described previously [6]. Briefly, after being deprived of food for 24 h with free access to distilled water, rats were fastened on board after anesthetized with 10% chloral hydrate (0.30 mL/100 g *i.p.*), and laid flat. Then, a flexible catheter was carefully inserted into the colon and the tip was 8 cm proximal to the anus. To induce colitis, a solution of 0.8 mL of TNBS/ethanol (5% TNBS aqueous solution: 70% ethanol solution = 1:1) was instilled slowly into the lumen of the colon within 1 minute. After that, rats were carefully held upside down in a vertical position, and the catheter was kept in lumen of the rat colon for 1 minute before removed gently. Then, TNBS-treated rats were kept upside down in a vertical position for 10 minute to prevent the leakage of the intra colonic instill. In normal control group, rats were received 35% ethanol solution alone using the same technique. The TNBS-treated rats were randomly assigned to 5 groups with 10 rats each: model group, SASP (400 mg/kg) group and SPA (50, 100, 200 mg/kg) groups. The drugs were dissolved in distilled water and administered once daily by oral gavages. After 7 days, rats were sacrificed and the portion of colon was excised for histopathological examination and biochemical investigation.

Evaluation of colon damage by macroscopic scoring

Macroscopic damage was assessed by the scoring system of Wallace *et al* [19]. The criteria for assessing macroscopic damage and the numerical rating score on a 0-10 scale were as follows: grade 0: no macroscopic changes; grade 1: focal hyperemia, no ulcers; grade 2: ulcer without significant inflammation (hyperemia and bowel wall thickening); grade 3: ulceration with inflammation at one site; grade 4: two or more sites of ulceration/inflammation; grade 5: major sites of damage extending ≥ 1 cm along colon length; grade 6-10: when the area of damage exceeds 2 cm along the colon, the score was increased by one for each additional 1 cm.

Evaluation of colon damage by histopathologic scoring

The colonic tissues were fixed in 4% neutral formalin, dehydrated with increasing concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5 μm thick) were mounted on slides, cleared, hydrated and stained with hematoxylin and eosin. Histopathological grading system was used in a blinded manner [7]. Grade 0: histological findings identical to normal mice; Grade 1: mild mucosal and/or submucosal inflammatory infiltrate and edema, punctuate mucosal erosions often associated with capillary proliferation, muscularis mucosae intact; Grade 2: 50% of the specimen display Grade 1 changes; Grade 3: prominent inflammatory infiltrate and edema frequently with deeper areas of ulceration extending through the muscularis mucosa into the submucosa, rare inflammatory cells invading the muscularis propria but without muscle necrosis; Grade 4: 50% of the specimen display Grade 3 changes; Grade 5: extensive ulceration with coagulative necrosis bordered underneath by numerous neutrophils and lesser numbers of mononuclear cells, necrosis extends deeply into the muscularis mucosae; and Grade 6: 50% of the specimen display Grade 5 changes.

Determination of colon MPO activity

Colon tissues were homogenized in cold physiological saline. The MPO activity was determined with the *o*-dianisidine method using a MPO detection kit [8].

Measurement of inflammatory cytokines production by ELISA

Colon tissues were homogenized in phosphate buffer saline and centrifugated to get supernatant for determination. The protein concentration and the levels of TNF-α, IL-1β, IL-6 and INF-γ in colon homogenate supernatants were measured using BCA protein determination kits and ELISA kits, respectively [9]. The corresponding

Table 1. Primer sequences for real-time PCR

Gene	Primer sequences	Product length (bp)
TNF-α	Forward: 5'-CAGGTTCCGTCCCTCTCATA-3' Reverse: 5'-TGCCAGTTCCACATCTCG-3'	100
IL-1β	Forward: 5'-GCCAACAAGTGGTATTCTCCA-3' Reverse: 5'-CCGTCTTTCATCACACAGGA-3'	118
IL-6	Forward: 5'-AGTTGCCTTCTTGGGACTGA-3' Reverse: 5'-ACTGGTCTGTTGTGGGTGGT-3'	102
IFN-γ	Forward: 5'-GGTGAACAACCCACAGATCC-3' Reverse: 5'-CAGAATCAGCACCGACTCCT-3'	114
GAPDH	Forward: 5'-GACATGCCGCCTGGAGAAAC-3' Reverse: 5'-AGCCCAGGATGCCCTTTAGT-3'	92

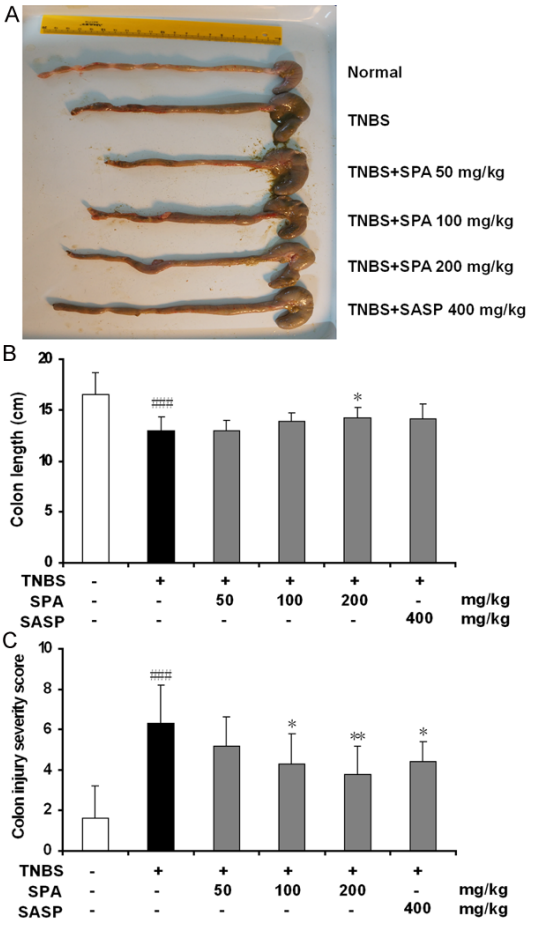

Figure 2. SPA attenuated TNBS-induced macroscopic damage of colon in rats. Experimental UC in rats was induced with TNBS/ethanol solution by instilling into the lumen of the colon. SPA (50, 100, 200 mg/kg) and SASP (400 mg/kg) were dissolved in distilled water and administered once daily by oral gavages. After 7 days, rats were sacrificed and the portion of colon was excised for morphological examination. A. Representative photograph of colon morphology of each group. B. The length colon. C. Macroscopic injury severity score of colon. Data are expressed as the means ± SD (n = 10). ###P<0.001 vs. control group; *P<0.05 and **P<0.01 vs. TNBS treated only group.

levels of inflammatory cytokines were expressed as pg/mg protein.

Measurement of inflammatory cytokines mRNA expressions by real-time PCR

Colon tissues were lysed with Trizol reagent and the total RNA was isolated according to the manufacturer's protocol. The total RNA was reverse-transcribed into cDNA by using oligo (dT) primers. Real-time PCR was performed using the SYBR Green PCR master mix. The primer sequences were shown in **Table 1**. The mRNA ex-pression levels of the tested genes relative to GAPDH were determined using the $2^{-\Delta\Delta Ct}$ method and expressed as fold induction [9].

Analysis of PPARγ expressions by Western blot

The relative protein levels of PPARγ in total protein of colon tissues were analyzed by Western blot. In brief, total proteins in colon tissues were extracted using RIPA buffer containing protease inhibitor cocktail and phosphatase inhibitor. The protein concentration was determined by a BCA protein determination kit. Equal amounts of denatured proteins were separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membrane. Immunoblotting was performed as described previously [10]. Blots were blocked with 5% skim milk for 2 h and then probed with primary antibodies (PPARγ and GAPDH) in 5% skim milk overnight at 4°C. IRDye 680LT secondary antibodies were dissolved in PBS and incubated with blots at room temperature for 2 h. Finally, the membranes were visualized using an Odyssey imaging system (LI-COR Inc., USA).

Statistical analysis

The data were expressed as mean ± standard deviation (SD) and examined for their statistical significance of difference with ANOVA and the standard's t-test. *P*-values of less than 0.05 were considered to be statistically significant.

Results

SPA attenuated TNBS-induced macroscopic damage of colon in rats

Based on the clinical usage of traditional Chinese medicine, *S. pubescens* was used by

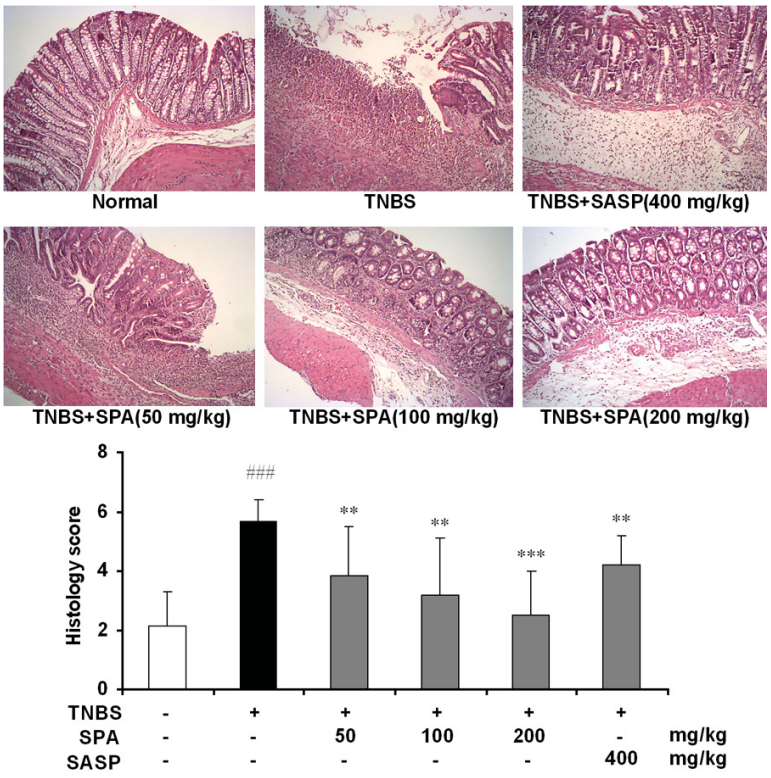

Figure 3. SPA attenuated TNBS-induced histopathologic damage of colon. Sections of the colon were stained with H&E for histopathological analysis. Representative histological photo (100 × magnification) and histological score of each group were presented. Data are expressed as the means ± SD (n = 10). ###*P*<0.001 vs. control group; ***P*<0.01 and ****P*<0.001 vs. TNBS treated only group.

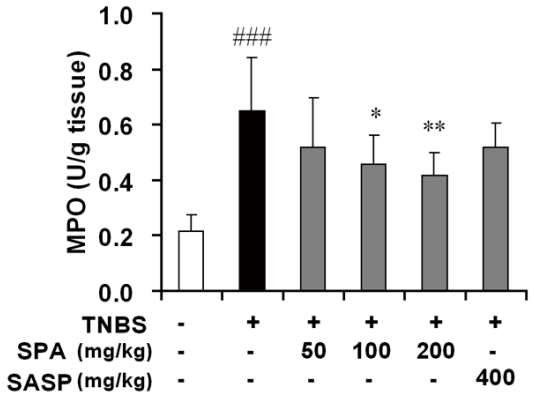

Figure 4. SPA inhibited TNBS-induced MPO accumulation in colon. Colon tissues were homogenized in cold physiological saline, the MPO activity was determined with the o-dianisidine method using a MPO detection kit. Data are expressed as the means ± SD (n = 10). ###*P*<0.001 vs. control group; **P*<0.05 and ***P*<0.01 vs. TNBS treated only group.

decoction with boiling water in clinic. Therefore, the aqueous extract (SPA) was used to evaluate the therapeutic effect of *S. pubescens* on TNBS-induced experimental UC in rats. TNBS elicits cell-mediated immune responses and induces transmural inflammation in the colons, which is characterized by increased leukocyte infiltration, edema and ulceration. These morphological and histopathological features resemble the acute phase in human UC [11]. In present study, the rats in normal control group showed slight diarrhea after 35% ethanol solution instillation, then switched back with normal appetite, shining fur, normal stool and no bloody diarrhea, and body weight steadily increased. In contrast, the rats in TNBS-induced model group showed less dynamic, listlessness, poor appetite, and the color of fur was coarse and lackluster. Some of them showed obvious bloody diarrhea. The weight body obviously decreased after TNBS instillation, and did not increase significantly even by the end of the experiment. SPA and positive control SASP were able to prevent the development of TNBS-induced these pathological changes. As shown in **Figure 2A** and **2B**, a significant decrease of the colon length of the rats, an indicator of inflammation, was found in TNBS-treated model rats. However, SPA at 200 mg/kg significantly attenuated the decrease of colon length induced by TNBS ($P<0.05$). The colon damage was further evaluated by macroscopic scoring. As shown in **Figure 2C**, TNBS caused colonic edema, hyperemia, inflammation and anabrosis, and a significant increase in the macroscopic injury severity score compared to normal control rats. Treatment with SPA (100 and 200 mg/kg) or SASP (400 mg/kg) significantly decreased both hyperemia and inflammation in the colons and reduced the macroscopic score compared to TNBS treated rats ($P<0.05$ or $P<0.01$).

SPA attenuated TNBS-induced histopathologic damage of colon

The histological observations also supported above results. As shown in **Figure 3**, compared

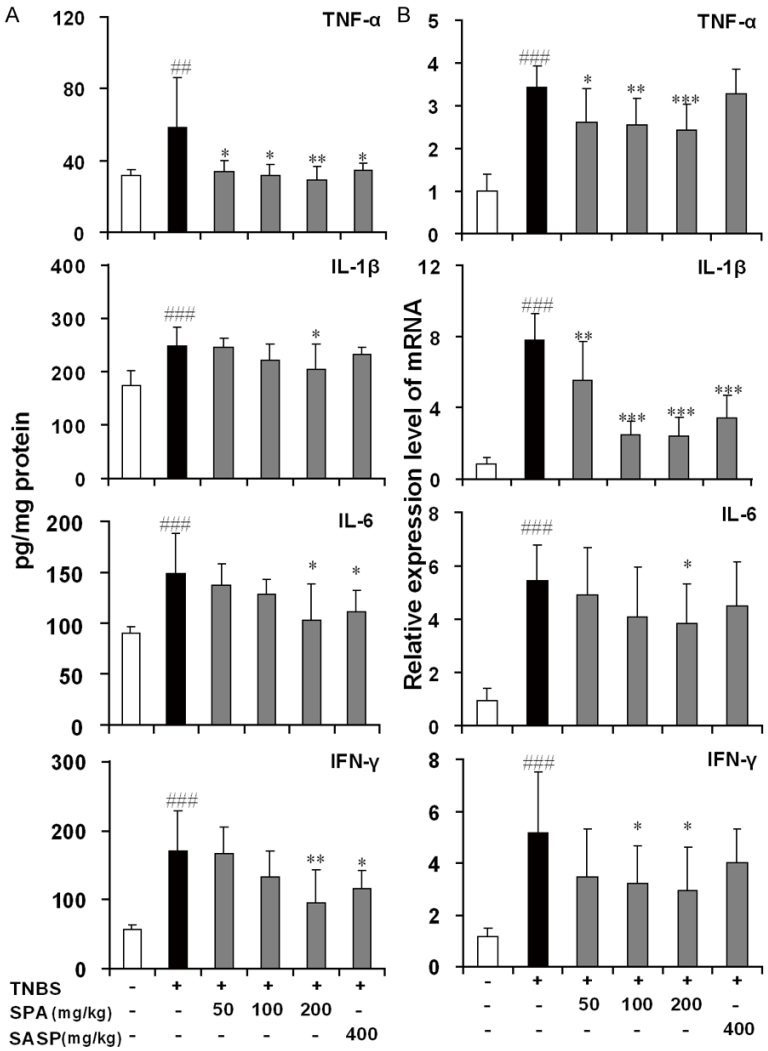

Figure 5. SPA inhibited TNBS-induced pro-inflammatory cytokines production in colon. A. Colon tissues were homogenized with phosphate buffer saline. The protein concentration and the levels of TNF-α, IL-1β, IL-6 and INF-γ in colon homogenate supernatants were measured using BCA protein determination kits and ELISA kits, respectively. The corresponding levels of pro-inflammatory cytokines were expressed as pg/mg protein. B. The total RNA was isolated from colon tissues and reverse-transcribed into cDNA. The mRNA expressions of pro-inflammatory cytokines relative to GAPDH were examined by Real-time PCR method and expressed as fold induction. Data are expressed as the means ± SD (n = 10). ###P<0.001 vs. control group; *P<0.05, **P<0.01 and ***P<0.001 vs. TNBS treated only group.

to normal control rats, TNBS-treated model rats showed massive necrotic destruction of epithelium and diffuse inflammatory cellular infiltration in the mucosa. The crypts were found to be distorted and there was loss of epithelium. However, treatment with SPA (50-200 mg/kg) or SASP (400 mg/kg) significantly attenuated the extent and severity of the histological signs of cell damage. The colonic mucosa showed ulcers in the process of healing, evolving to a more chronic inflammatory infiltrate, with mononuclear predominance and initiation of a repair process. The elevation of microscopic pathological score induced by TNBS was significantly decreased by SPA or SASP treatment (P<0.01).

SPA inhibited TNBS-induced MPO accumulation in colon

MPO is abundantly expressed in neutrophil and its activity reflects the degree of neutrophil infiltration, which is a marker of acute inflammation [12]. The results (**Figure 4**) showed that TNBS significantly caused the increase of MPO levels in the colonic tissues, and this increase could be inhibited by SPA (100 and 200 mg/kg) obviously (P<0.05 or P<0.01).

SPA inhibited TNBS-induced pro-inflammatory cytokines production in colon

TNF-α, IL-1β, IL-6 and IFN-γ are predominant pro-inflammatory cytokines in human UC. These cytokines play key roles in the initiation, augmentation and perpetuation of the disease, since they are directly responsible for the mucosal injury [13]. In this study, the concentrations of TNF-α, IL-1β, IL-6 and IFN-γ in the colonic tissues were detected by ELISA method. The results were shown in **Figure 5A**, TNBS significantly increased the concentrations of TNF-α, IL-1β, IL-6 and IFN-γ in the colonic tissues, and these increases were inhibited by SPA or SASP obviously (P<0.05 or P<0.01). In order to further verify the inhibitive effects of SPA on pro-inflammatory cytokines, real-time PCR was performed to determine the mRNA expression using specific primers, and the results were

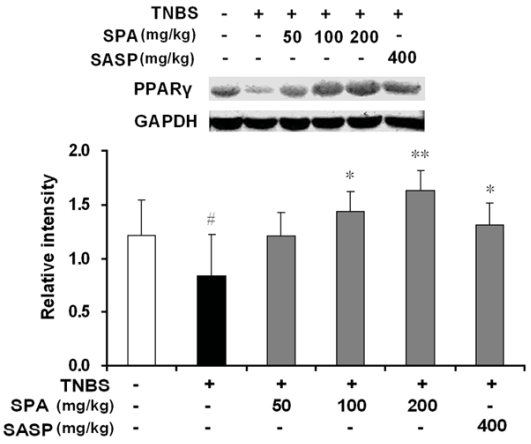

Figure 6. SPA restored the expression of PPARγ in TNBS-impaired colon. The protein levels of PPARγ in total protein of colon tissues were analyzed by Western blot and expressed as relative intensity to GAPDH. Data are expressed as the means ± SD (n = 10). #*P*<0.05 vs. control group; **P*<0.05 and ***P*<0.01 vs. TNBS treated only group.

shown in **Figure 5B**. The TNBS-induced elevation of mRNA expression levels of these four cytokines were suppressed by SPA or SASP treatment significantly (*P*<0.05 or *P*<0.01).

SPA restored the expression of PPARγ in TNBS-impaired colon

PPARγ is a nuclear receptor which acts directly to negatively regulate gene expression of pro-inflammatory genes in UC [14]. Therefore, to explore the mechanism underlying the anti-inflammatory activities of SPA, the expression of PPARγ in colon was assayed by Western blot. As shown in **Figure 6**, TNBS prominently decreased the expression of PPARγ in the colonic tissues, and these decreases were significantly restored by SPA (100 and 200 mg/kg) or SASP (400 mg/kg) (*P*<0.05 or *P*<0.01).

Discussion

In previous studies, the extract of *S. pubescens* by 50% (v/v) ethanol-water was reported to have therapeutic effects on an osteoarthritis rabbit model by prominent cartilage protection through controlling aggrecanase, MMPs/TIMP-1 levels, and inflammatory mediators (IL-1β, PGE2 and NO) [15]. Topical application of the methanolic extract of *S. pubescens* displayed wound healing activity in rats and anti-inflammatory activity in carrageenan-induced edema rats [16, 17]. Kim has reported that oral administration of the aqueous extract of *S. pubescens* significantly inhibited immunoglobulin E (IgE)-mediated immediate hypersensitivity reaction in rats and inhibited histamine release from rat peritoneal mast cells [18]. In clinic of traditional Chinese medicine, *S. pubescens* was often used by decoction with boiling water for the treatment of inflammatory and autoimmune diseases [3]. Thus, in our present study, *S. pubescens* was extracted with boiling water, and oral administration of this aqueous extract (SPA) was showed to ameliorate colonic edema, hyperemia, inflammation, anabrosis and MPO production in TNBS-induced experimental UC in rats. These results further corroborated the anti-inflammatory activity of *S. pubescens* and suggested the potential of this traditional Chinese medicine as an agent for UC.

PPARγ was originally identified as a ligand-activated nuclear receptor expressed in adipose tissue, where it controls the lipid metabolism and insulin sensitization. The colon has also been identified as one of the tissues expressing the highest levels of PPARγ, next to adipose tissue in epithelial cells and, to a lesser degree, macrophages and lymphocytes [19]. It was found that PPARγ was down-regulated in the colon of UC patients compared with healthy control individuals and the expression of PPARγ was significantly associated with disease activity [20, 21]. Impaired epithelial expression of PPARγ has also been confirmed in various experimental animal model of colitis induced by dextran sodium sulfate (DSS), TNBS or ischemia-reperfusion injury [22-24]. The aetiology underlying impaired PPARγ expression in colonic epithelial cells of UC patients is incompletely understood, but it is definite that perturbed levels of PPARγ in UC are not secondary to the inflammatory, and accumulating evidences have recognized PPARγ as an endogenous negative regulator of intestinal inflammation, playing an important role in the pathogenic process of UC [14]. PPARγ interferes with inflammatory pathways by interactions with transcription factors such as nuclear factor kappa B (NF-κB), activating protein-1 (AP-1), signal transducer and activator of transcription (STAT), and nuclear factor-activated T cell (NFAT). Activated PPARγ directly binds corepressors of transcription factors, making them unfit for nuclear translocation and transcription, thereby block

the expression of inflammatory mediators, such as IL-1β, COX-2, IL-6, IL-8, TNF-α, IFN-γ, iNOS, and chemokines [25]. PPARγ$^{+/-}$ heterozygous mice were susceptible to TNBS-induced inflammation [23]. PPARγ agonists (troglitazone, rosiglitazone, 5-ASA, et al) decreased colitis severity in different animal models of colitis induced by chemical compounds (DSS and TNBS), ischaemia, bacteria, or genetically modified animals [25]. Moreover, in a randomized multicenter, double-blind, placebo-controlled clinical trial, clinical response occurred in 23 patients (44%) treated with rosiglitazone and 12 patients (23%) treated with placebo (P = 0.03). Clinical remission was achieved in 9 patients (17%) treated with rosiglitazone and 1 patient (2%) treated with placebo (P = 0.01) [26]. All of these evidences suggested that PPARγ targeting is of therapeutic interest in the treatment of UC.

In the past few years, phytochemical investigations revealed that diterpenoids and flavonoids are the main bioactive constituents in *S. pubescens* [27-31]. These constituents in other plants have been demonstrated to mediate anti-inflammatory effects through activating PPARγ [32, 33]. Therefore, to investigate the mechanism underlying the therapeutic effects of SPA on UC, present studies further detected the productions of pro-inflammatory cytokines and the expression of PPARγ in TNBS- impaired colonic tissues. The results showed that TNBS significantly increased the production pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IFN-γ) and decreased the expression of PPARγ in the colonic tissues, which are consistent with the studies by Soubh et al [34]. SPA not only inhibited pro-inflammatory cytokines production, but also up-regulated PPARγ expression in colon. These findings indicated that SPA prevented TNBS-induced UC by inhibiting inflammatory mediators through PPARγ, although it is not clear that this is the only mechanism by which SPA acts.

Previous studies have reported the anti-inflammatory activities of the chemical constituents in *S. pubescens*. Wang *et al* reported that several sesquiterpenoids and diterpenoids from this plant inhibited NO production in activated RAW 264.7 macrophages and FMLP/CB induced $O_2(\cdot-)$ generation and elastase release in human neutrophils [35]. Lee *et al* also reported that ent-kaurane and ent-pimarane diterpenes inhibited lipopolysaccharide-induced NO production and the expression of iNOS and COX-2 in BV2 microglia [29]. Kirenol attenuated synovial inflammation of collagen-induced arthritis in rats. Siegeskaurolic acid inhibited iNOS and COX-2 expression in RAW 264.7 macrophages. The anti-inflammatory mechanisms of these two main constituents in *S. pubescens* are through interacting with NF-κB [36, 37]. We conjectured that these bioactive constituents may responsible the therapeutic effect of *S. pubescens* on UC in present study. However, it is poorly understood whether the anti-inflammatory mechanisms of these bioactive constituents are through modulating PPARγ, which need to be confirmed in future experiments. Kim *et al* have found that three ent-kaurane diterpenes from *Acanthopanax koreanum* up-regulated PPARγ and PPARβ/δ activities, whereas they did not activate PPARα activity [32]. These findings imply the potential of identifying novel selective PPARγ modulators from diterpenes of *S. pubescens*. These works are currently being carried out in our laboratory.

Conclusion

In summary, it was concluded that SPA has therapeutic effects against TNBS-induced experimental UC in rats, and its underlying mechanisms involve the modulation of PPARγ-mediated production of inflammatory mediators. These findings highlight the potential of this traditional Chinese medicine as an agent for use in the treatment of UC.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

MPO, Myeloperoxidase; PPARγ, peroxisome proliferator-activated receptor gamma; SASP, sulfasalazine; SPA, the aqueous extract of Siegesbeckia pubescens; TNBS, trinitrobenzene sulfonic acid; UC, ulcerative colitis.

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