Original Articles

Acupuncture and Herbal Medicine

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Wei Chang An pill alleviates 2,4,6-trinitro-benzenesulfonic acid-induced ulcerative colitis by inhibiting epithelial-mesenchymal transition process

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Abstract

Objective: To investigate the inhibitory effect and mechanism of Wei Chang An pill (WCA) on ulcerative colitis (UC).

Methods: A 2,4,6-trinitro-benzenesulfonic acid (TNBS)-induced UC model was established, and WCA was administered orally for 1 week. Body weight, colon length, disease activity index (DAI) score, and colon mucosa damage index (CMDI) score were recorded. Cytokine expression in lipopolysaccharide (LPS)-stimulated THP-1 cells was evaluated to determine the anti-inflammatory effects of WCA and its active ingredients. Immunohistochemistry and immunofluorescence were performed to detect the expression of epithelial-mesenchymal transition (EMT) markers E-cadherin and vimentin in rat UC and WCA groups, and in Caco-2 cells stimulated with conditioned medium (CM) from THP-1 cells, with or without LPS or WCA.

Results: WCA significantly inhibited body weight loss, decreased DAI and CMDI scores, blocked colon length shortening, and improved histological damage in UC rats. Furthermore, both myeloperoxidase (MPO) activities and cytokine expression in UC tissues were significantly suppressed by WCA as well. In THP-1 cells, the mRNA expression of interferon-inducible protein (IP)-10, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and NF-κB inhibitor α (IκBα) was significantly suppressed by WCA and its active ingredients. E-cadherin expression in UC rats and CM-stimulated Caco-2 cells was downregulated and vimentin expression was upregulated, whereas both were blocked when administered with WCA.

Conclusions: Our data showed that WCA alleviated UC progression by inhibiting inflammation-induced EMT progression.

Keywords: Epithelial-mesenchymal transition, Inflammation, Traditional Chinese medicine, Ulcerative colitis, Wei Chang An

Graphical abstract: http://links.lww.com/AHM/A56.

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Acupuncture and Herbal Medicine (2023) 3:2

Received 31 May 2022 / Accepted 27 March 2023

http://dx.doi.org/10.1097/HM9.0000000000000064

Introduction

Ulcerative colitis (UC) is an immune-mediated inflammatory bowel disease with high morbidity[1–2]. The prevalence of UC ranged from 7.6 to 245 cases per 100,000 persons/year[3] in 2004, and is expected to reach 30 million by 2025[4]. Typical UC symptoms include diarrhea, rectal urgency, tenesmus, and abdominal pain to variable degrees, which seriously affect the quality of life of patients[5]. Previous studies have shown that during UC development, a large number of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-18, and IL-1β, are highly expressed in macrophages and secreted into the microenvironment, which may lead to a serious inflammatory status[6–7]. Epithelial-mesenchymal transition (EMT) is an essential phenotypic conversion in the epithelial cells loss intercellular adhesion and acquires fibroblast-like characteristics[8], which has also been identified as an important feature of UC[9].

Immunomodulators or biological agents, such as 5-aminosalicylic acid (5-ASA) drugs, corticosteroids, calcineurin inhibitors, or tumor necrosis factor-α antibodies, are often used to treat patients with moderate or severe UC[10–12]. However, these biological agents cause severe damage due to autoimmunity and produce side effects, such as liver and kidney damage and protein metabolism disorders[11]. The Wei Chang An pill (WCA)

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is a traditional Chinese herbal preparation that consists of ten Chinese medicinal herbs, including *Aquilaria sinensis* (Lour.) Gilg (Thymelaeaceae), *Citrus aurantium* L. (Rutaceae) (Fructus Aurantii), *Magnolia officinalis* Rehd. et Wils. (Magnoliaceae), *Santalum album* L. (Santalaceae), *Rheum offificinale* Baill. (Polygonaceae), *Croton tiglium* L. (Euphorbiaceae), *Moschus moschiferus* Linnaeus, *Ligusticum chuanxiong* Hort. (Umbelliferae), and *Ziziphus jujuba* Mill. (Rhamnaceae)[13]. Based on theory, WCA has positive effects on removing turbidity and dampness, promoting the circulation of *qi*, relieving pain, invigorating the spleen, and improving stagnation[13]. Clinically, WCA is widely applied for the treatment of various gastrointestinal diseases, such as diarrhea, enteritis, dysentery, irritable bowel syndrome, nausea, vomiting, indigestion, and UC[14–15]. However, few studies have reported on the mechanism of WCA in UC treatment. Only one study found that WCA improved the inflammatory response in dextran sodium sulfate-induced UC mice by regulating regulatory T cells and T helper 17 cells[16].

In this study, we investigated the anti-inflammatory effect of WCA *in vivo* using 2,4,6-trinitro-benzenesulfonic acid (TNBS)-induced UC rat models and lipopolysaccharide (LPS)-induced monocyte THP-1 cells. EMT was detected in UC tissues and colorectal adenocarcinoma Caco-2 cells in an inflammatory environment, and the effects of WCA on the EMT process were further evaluated. Our findings provide evidence that WCA contributes to the inhibition of UC by suppressing inflammation-induced EMT, indicating that WCA is a potential candidate for UC therapy.

Materials and methods

Chemicals and reagents

Atractylodin (ATL, CAS:55290-63-6) and nootkatone (NK, CAS:4674-50-4) were purchased from Chengdu Aifabiotech Co., Ltd. (Chengdu, China). Costunolide (Cos, CAS:553-21-9), betulinic acid (BA, CAS:472-15-1), chlorogenic acid (ChA, CAS:327-97-9), dehydrocostus lactone (DHL; CAS:477-43-0), β-caryophyllene (BCP, CAS:87-44-5), and benzyl acetone (Ben, CAS:2550-26-7) were obtained from Victory Biological Technology Co., Ltd. (Sichuan, China). TNBS (P2297) and LPS from *Escherichia coli* 055:B5 (L6529) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).WCA was supplied by Le Ren Tang Pharmaceutical Factory, Tianjin Zhongxin Pharmaceutical Group Co., Ltd. (batch no. 1530819; Tianjin, China). Sulfasalazine (SASP) was produced by Shanghai Fudan Pharmaceutical Co., Ltd. (batch no. 22200103; Shanghai, China). Fecal occult blood kits were purchased from Beijing Leagene Biotechnology Co., Ltd. (cat no. TC0513). Myeloperoxidase (MPO) kit was purchased from Wuhan Huamei Bioengineering Co. Ltd (cat no. CSB-E08722r; Wuhan, China). IL-6 (cat no. JYM0646Ra), TNF-α (cat no. JYM0635Ra), and IL-1β (cat no. JYM0419Ra). Enzyme linked immunosorbent assay (ELISA) kits were purchased from Wuhan Colorful Gene Biological Technology Co., Ltd. (Wuhan, China) Anti-E-cadherin Mouse mAb were purchased from Wuhan Servicebio Biotechnology Co., Ltd. (cat no. GB12082; Wuhan, China). Rabbit Polyclonal Anti-Vimentin Antibody was purchased from Santa Cruz Biotechnology, Inc. (sc-6260; Dallas, TX, USA). The cell culture reagents and supplies were purchased from Biological Industries (Beijing, China).

Animals

Male Wistar rats (280 ± 10) g were supplied by Beijing Charles River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were kept under standard conditions at ambient temperature (24 ± 2) °C in a 12-h light-dark cycle and given access to food and water. All rats were acclimatized for 1 week prior to the experiments. The experimental procedures were approved by the Tianjin University of Traditional Chinese Medicine Animal Research Committee (TCM-LAEC20170018), in accordance with the Guide for the Care and Use of Laboratory Animals.

TNBS-induced UC in rats

First, the rats were randomly divided into six groups (n = 6): control group, UC group, WCA-treated groups with low (22 mg/kg, WCA-L), medium (44 mg/kg, WCA-M) and high (88 mg/kg, WCA-H) doses, and SASP group (100 mg/kg). The rats were fasted for 24 h and then anesthetized with pentobarbital sodium (0.1 g/kg). Except for the control group, the rats in all other five groups were administered TNBS at a dose of 100 mg/kg by injecting into the colon approximately 8 cm from the proximal to the anus using a polyvinyl catheter 2 mm in diameter, and subsequently maintained in a head-down position for 10 min to induce UC. In the control group, rats were treated with physiological saline at an equal volume instead of the TNBS solution. After 24 h, the rats were orally administered WCA or SASP daily at the indicated doses for 7 d.

Daily observations and sample collection

The rats were observed and the observations were recorded daily for general appearance, such as body weight, stool consistency, and rectal bleeding. The disease activity index (DAI) score was evaluated according to the standards shown in Table 1[17]. Twenty-four hours after the last drug administration, the rats were anesthetized, and blood samples were collected from the abdominal aorta. Colon tissue was collected, and the length (between the proximal rectum and the ileocecal junction) was measured when the rats were euthanized. Visible colonic lesions (1 cm) were collected for

Table 1

The scores for DAI

DAI score	Weight loss (%)	Stool consistency	Occult/gross bleeding
0	None	None	None
1	1–5	-	-
2	5–10	Loose stools	Hemoccult+
3	10–15	-	-
4	>20	Diarrhea	Gross bleeding

DAI: Disease Activity Index.

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histopathological staining. The remaining colon tissues were stored at −80°C until further analysis.

Assessment of colon mucosa damage index

According to the extent of ulceration, inflammation, and wall thickening, the colon mucosa damage index (CMDI) score was assessed with scores ranging from 0 to 10, as described in Table 2[18].

Histopathological examination

The proximal and distal colon were used for histological analysis. Tissues were first fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, and cut into 5-μm-thick sections. Hematoxylin and eosin (H&E) staining was performed as previously reported[19]. The tissues were examined under a microscope (Nikon, Tokyo, Japan) in random order.

Measurement of MPO activity

Rat blood was centrifuged at 2,000×*g* for 15 min at 4°C, and the supernatants were collected for the measurement of MPO activity using an MPO kit. All procedures were performed according to the manufacturer's instructions.

Enzyme linked immunosorbent assay

The levels of IL-6, TNF-α, and IL-1β in rat sera were assessed using the ELISA kit according to the manufacturer's protocol.

Cell culture

Human monocyte THP-1 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 UI/mL penicillin, and 100 mg/mL streptomycin in a 5% CO_2 temperature at 37°C. Caco-2 cells were cultured in minimum essential medium (MEM) supplemented with 20% FBS, under the same conditions. THP-1 and Caco-2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA).

Preparation of conditioned medium

THP-1 cells were seeded into a 6-well plate at a density of 3×10^6/well, pretreated with WCA (88 μg/mL), and incubated for 4 h, followed by stimulation with LPS (100 ng/mL). After incubating for an additional 48 h, the culture medium was collected, centrifuged, and stored at −80°C in conditioned medium (CM) for further experiments.

Table 2

The scores for CMDI

Score	Mucosal morphology
0	No ulcers and inflammation
1	Focal congestion, no ulcer
2	Ulcer without congestion or thickening of the intestinal wall
3	One place with inflammatory ulcer
4	Ulcers and inflammatory sites in two places
5	The main part of the damage along the colon extension ≥1 cm
6–10	Injury along the length of the colon extended ≥2 cm

CMDI: Colonic mucosa damage index.
(1) For each increase of 1 cm damage, the score increased by 1 point; (2) The presence of adhesion: 0 points, normal; 1 point, slight adhesion; 2 points, the main adhesion.

Quantitative reverse transcription polymerase chain reaction

THP-1 cells were pretreated with WCA (88 μg/mL) or its active ingredients (10 μM) and incubated for 4 h, followed by LPS stimulation to induce inflammation. After incubating for an additional 16 h, the total RNA from cells was extracted using TRIzol reagent (CWBIO) according to the manufacturer's protocol. AT341-01 was used to generate complementary DNA (cDNA). The mRNA expression levels of *IP-10*, *IL-6*, *TNF-a*, *IL-1β*, and *IκBα* were detected using the GoTaq-qPCR-Master-Mix [A6001, Promega (Beijing) Biotech Co., Ltd.]. The relative gene expression was normalized to that of GAPDH using the $2^{-\Delta\Delta Ct}$ method. The polymerase chain reaction (PCR) primer sequences used are listed in Table 3.

Immunofluorescence assay

Caco-2 cells were seeded at a density of 2.5×10^4 cells/well in 24-well plates. After cell attachment, the medium was replaced with CM and the cells were cultured for an additional 7 d. Next, Caco-2 cells were fixed in 4% formaldehyde 4°C pre-cooled methanol at −20°C, followed by blocking with the 2% bovine serum albumin (BSA). Thereafter, the cells were incubated with primary antibodies against E-cadherin (#5296; Cell Signaling, MA, USA) and vimentin (sc-6260; Santa Cruz Biotechnology Inc.) at 4°C overnight. On the second day, the cells were incubated with FITC-mouse goat secondary antibody (#F2761; Invitrogen, Shanghai, China). The nuclei were stained with DAPI (S2110; Solarbio, Beijing, China). Images were captured using a Perkin Elmer High content

Table 3

Primers for real-time PCR

Species	Gene	Primer sequences (5′–3′)
Rats	*TNF-α*	F: ATGGGCTCCCTCTCATCAGT
		R: GCTTGGTGGTTTGCTACGAC
	IL-1β	F: CTTTGAAGAAGAGCCCGTCC
		R: CCAAGGCCACAGGGATTTTG
	GAPDH	F: ATGATTCTACCCACGGCAAG
		R: CTGGAAGATGGTGATGGGTT
	IL-18	F: GCCATGTCAGAAGAAGGCTCT
		R: GGATTCGTTGGCTGTTCGGT
	IL-6	F: ACC ACC CAC AAC AGA CCA GT
		R: TTT CAC AGC CTA CCC ACC TC
Human	*GAPDH*	F: ATGATTCTACCCACGGCAAG
		R: CTGGAAGATGGTGATGGGTT
	IP-10	F: GGTGAGAAGAGATGTCTGAATC
		R: GTAGGGAAGTGATGGGAGAG
	IκBα	F: GCACCTCCACTCCATCCTGAAGG
		R: CCATTACAGGGCTCCTGAGCATTG
	IL-6	F: GGCACTGGCAGAAAACAACC
		R: GCAAGTCTCCTCATTGAATCC

GAPDH:glyceraldehyde-3-phosphate dehydrogenase; IκBα:inhibitor of NF-κb; IL: Interleukin; IP: Inducible protein; PCR: Polymerase chain reaction; TNF-α: Tumor necrosis factor-α.

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screening system [Operetta, PerkinElmer Enterprise Management (Shanghai) Co., Ltd.; 20× magnification].

Immunohistochemical staining

To detect E-cadherin and vimentin expression, rat colon tissues were deparaffinized and rehydrated. Next, sodium citrate buffer was applied to retrieve the antigen, followed by blocking with 3% BSA. Thereafter, the tissues were incubated with primary antibodies against E-cadherin and vimentin overnight at 4°C. On the second day, the tissues were incubated with a secondary antibody at room temperature (25°C) for 2 h α, Along with TNF. Finally, sections were observed under a light microscope (Nikon, Tokyo, Japan; 100× magnification). The immunofluorescence (IF) staining of UC tissue was similar.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) of at least three independent experiments. Student's t test was performed to compare the differences between the two groups, and one-way ANOVA was used to analyze the data. All statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software Inc, San Diego, CA, USA), and the statistical significance was set at $P < 0.05$.

Results

WCA inhibited TNBS-induced UC in rats with a dose-dependent manner

As shown in Figure 1A, compared with the control group, the body weight in the UC group dropped significantly on the fourth day [(258.5 ± 15.86) g; $P < 0.05$)]. The DAI (2 days, 3.19 ± 0.36; $P < 0.05$) and CMDI scores (3.5 ± 0.84; $P < 0.01$) were markedly increased in the UC group (Figure 1B, C). Consistently, the length of the colon was noticeably shortened in the UC group (12.12 ± 0.84 and 7.57 ± 1.41; $P < 0.01$; Figure 1D, Supplementary Figure S1, http://links.lww.com/AHM/A55), In contrast, after the administration of WCA, the UC symptoms

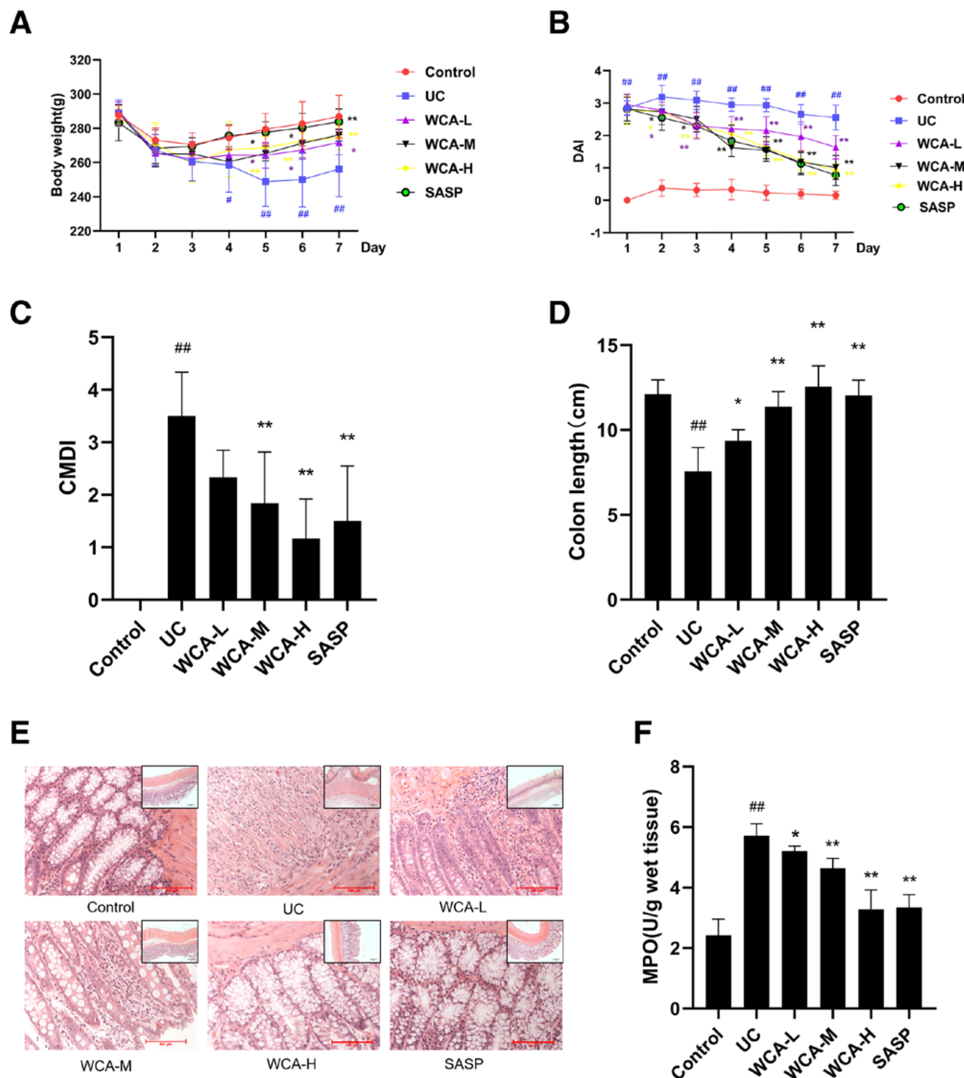

Figure 1. Effects of WCA administration on TNBS-induced UC in rats. Compared with the TNBS-treated control group, WCA reversed the decreased body weight (A), decreased the DAI (B) and reduced the increased CMDI (C) as well as colon length (D) and histologic injury (E). Images were taken at 40× magnification (scale bar: 100 μm) and 200× magnification (scale bar: 100 μm). (F) MPO activity in TNBS-treated rats. All data are expressed as the mean ± SD (n = 6). $^{\#\#}P < 0.01$ versus control group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus UC group. CMDI: Colon mucosa damage index; DAI: Disease activity index; MPO: Myeloperoxidase; SASP: Sulfasalazine; SD: Standard deviation; TNBS: 2,4,6-trinitro-benzenesulfonic acid; UC, Ulcerative colitis; WCA: Wei Chang An pill.

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were significantly alleviated in a dose-dependent manner, when compared with those in the UC group.

Histological analysis showed that in the control group, smooth mucosa of the colon was easily observed, and epithelial cells around the glands were arranged regularly. However, in the UC group, the goblet cells were damaged, the structure of the crypt faded away, and a large number of inflammatory cells infiltrated the colonic mucosa, submucosa, and muscular layers. WCA administration effectively restored crypt architecture and reduced histological inflammation compared with that in the UC group (Figure 1E).

MPO activity is an important marker of neutrophil granulocyte infiltration[20]. As shown in Figure 1F, MPO activity was markedly elevated in the UC group compared with that in the control group ($P < 0.01$). However, treatment with WCA significantly inhibited MPO activity in a dose-dependent manner ($P < 0.05$ and $P < 0.01$, respectively). Next, the levels of TNF-α, IL-6, IL-18, and IL-1β in rat colon tissues were detected, and the data showed that they were significantly increased in the UC group compared to those in the control group ($P < 0.01$) and were downregulated after WCA administration in a dose-dependent manner ($P < 0.05$ and $P < 0.01$; Figure 2). Furthermore, the levels of pro-inflammatory cytokines in the WCA-H group were significantly decreased and were comparable to those in the SASP group.

WCA and its herb active ingredients inhibited LPS-induced cytokines expression in THP-1 cells

The anti-inflammatory effects of WCA and its active ingredients were investigated using LPS-stimulated THP-1 cells. As shown in Figure 3, LPS significantly increased the mRNA expression of *IP-10*, *TNF-α*, *IL-6*, and *IκBα* in THP-1 cells ($P < 0.01$). However, these effects were completely blocked by WCA pre-treatment (Figure 3A).

Furthermore, the effects of its active ingredients on the LPS-induced expression of *IP-10*, *TNF-α*, *IL-6*, and *IκBα* were investigated. BA markedly suppressed *IP-10* and *IL-6* expression (Figure 3D), which also decreased *IP-10* and *TNF-α* expression (Figure 3E). β-BCP downregulated *IP-10* expression ($P < 0.05$; Figure 3G), and ChA distinctly suppressed *IP-10*, *TNF-α*, and *IL-6* expression ($P < 0.05$; Figure 3I). In addition, these cytokines were slightly influenced by ATL and Ben administration (Figure 3B, F). Taken together, the selective inhibitory effects of various active ingredients from WCA on LPS-targeted gene regulation were observed, which together may contribute to the anti-inflammatory effect of WCA.

WCA inhibited EMT in TNBS-induced UC rats and LPS-stimulated THP-1 cells induced Caco-2 cells

Given that both E-cadherin and vimentin are expressed in colon tissues, we examined their alterations under various conditions using IF and immunochemistry. According to the results (Figure 4), E-cadherin was expressed in the colon epithelial cells of the control group but was downregulated in the UC group. Vimentin was mainly expressed in stromal cells, and its expression was significantly increased in colon tissues in the UC group compared with that in the control group. The changes in these two indicators suggest the occurrence of EMT in the UC model. However, after the administration of WCA, the expression of E-cadherin increased and the expression

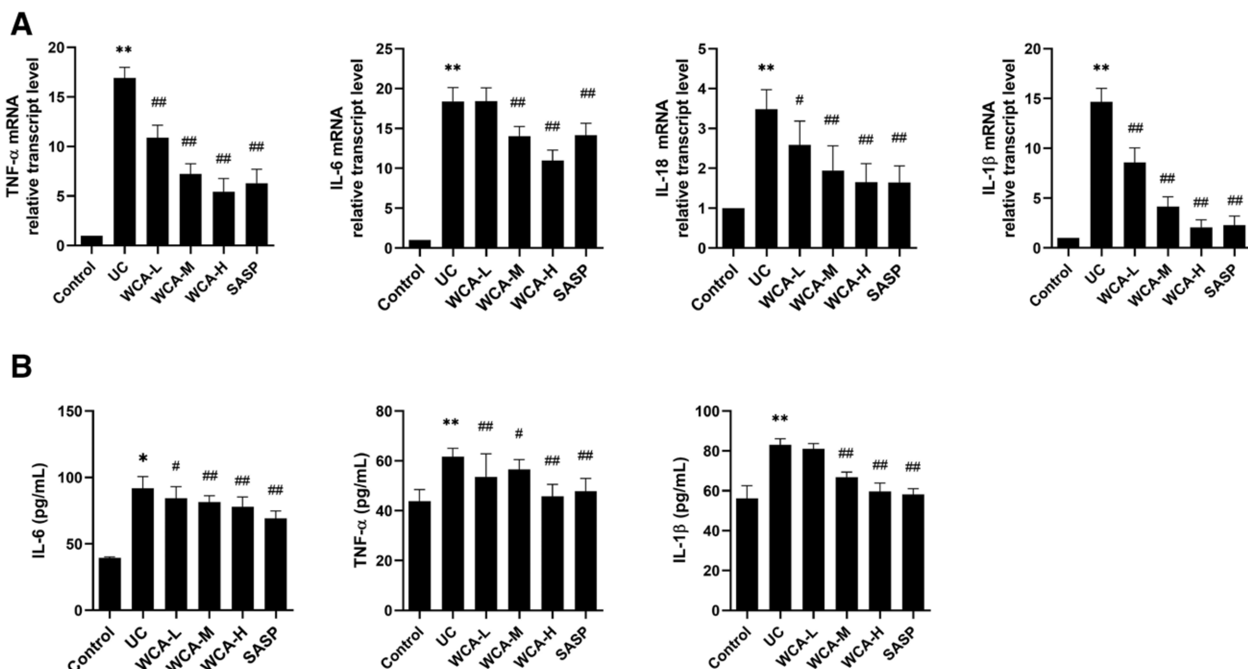

Figure 2. WCA-treated suppression of inflammatory in TNBS-induced UC in rats. The mRNA levels of (A) *TNF-α*, *IL-6*, *IL-18*, and *IL-1β* in colon tissues was detected with the RT-PCR. (B) Expression of IL-6, TNF-α, and IL-1β was determined with the ELISA. Data are expressed as the mean ± SD ($n = 6$). ##$P < 0.01$ versus control group. *$P < 0.05$, **$P < 0.01$ versus UC group. ELISA: Enzyme linked immunosorbent assay; IL: Interleukin; RT-PCR: Reverse transcription polymerase chain reaction; SASP: Sulfasalazine; SD: Standard deviation; TNBS: 2,4,6-trinitro-benzenesulfonic acid; TNF-α: Tumor necrosis factor-α; UC, Ulcerative colitis; WCA: Wei Chang An pill.

of vimentin significantly decreased, indicating that WCA inhibited the EMT process in TNBS-induced UC rats.

LPS-stimulated THP-1 cells secreted factors that produce an inflammatory microenvironment. In our study, we collected LPS-stimulated THP-1-derived CM to induce EMT in Caco-2 cells. As a control, the direct effects of LPS, with or without WCA, were investigated. As shown in Figure 5A, no obvious changes in E-cadherin or vimentin expression were observed in Caco-2 cells upon direct stimulation with LPS. However, when Caco-2 cells were incubated with LPS-stimulated THP-1 cell-derived CM, vimentin upregulation and E-cadherin downregulation were markedly amplified (Figure 5). After treatment with WCA, the abnormal levels of E-cadherin and vimentin were blocked.

Discussion

Traditional Chinese medicines (TCM) are a major group of drug candidates used to suppress inflammatory responses. Extensive efforts have been made to identify herbs with anti-inflammatory activity. In our study, we found that WCA had a significant anti-inflammatory effect on colonic inflammation in TNBS-induced UC rats (Figure 1). Furthermore, the anti-inflammatory effect of WCA was demonstrated to inhibit cytokine expression in UC rats (Figure 2) and LPS-induced THP-1 cells (Figure 3).

Radix Aucklandiae (RA) and *Lignum Aquilariae Resinatum (LAR)* are two key herbs considered to have a fundamental role in the effect of WCA in gastrointestinal diseases[21–22]. Herein, we evaluated the anti-inflammatory effects of the active ingredients in RA and LAR, and found that the inhibitory effects of ATL and Ben on cytokine expression were not significant when compared to stimulation with LPS alone in THP-1 cells. However, other ingredients significantly inhibited LPS-induced *IP-10*, *TNF-α*, *IL-6*, and *IκBα* expression (Figure 3). Previous studies have shown that ATL does not inhibit the interaction of NF-κB with the IL-6 promoter[23], indicating an independent mechanism of inactivation of LPS-related signals. It has also been reported that BA, BCP, and ChA exhibit anti-inflammatory effects in TNBS-induced UC rats and DSS-induced UC mice[24–26]. The other two ingredients, Cos and DHL, reduce inflammation and improve colorectal barrier function in UC mice[27–28]. Although there are no relevant reports on the inhibition of UC by NK, its anti-inflammatory effect has been found in lung injury and testicular toxicity[29–30].

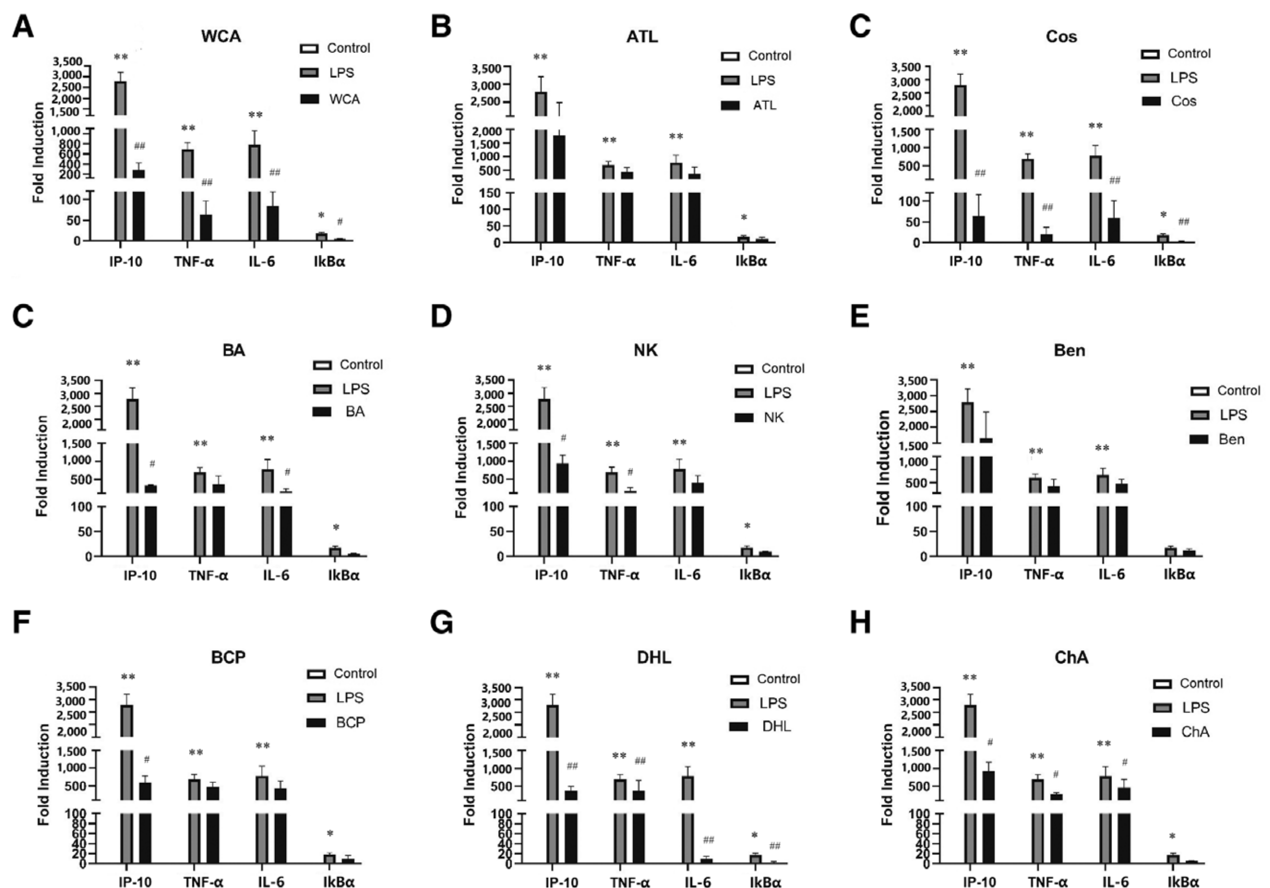

Figure 3. WCA and active ingredients isolated from WCA inhibited cytokine expression in THP-1 cells. (A) WCA, (C) Cos, and (H) DHL significantly decreased the high expression of IP-10, TNF-α, IL-6, and IκBα in THP-1 cells. (D) BA decreased the high expression of IP-10 and IL-6 in THP-1 cells. (E) NK decreased the high expression of IP-10 and TNF-α in THP-1 cells. (G) BCP decreased the high expression of IP-10 in THP-1 cells. (I) ChA decreased the high expression of IP-10, TNF-α, and IL-6 in THP-1 cells. (B) ATL and (F) Ben were not significantly inhibited by the expression of IP-10, TNF-α, IL-6, and IκBα in THP-1 cells. Data are expressed as the mean ± SD (n = 3). ##P < 0.01 vs control group. *P < 0.05, **P < 0.01 versus LPS-stimulated group. ATL: Atractylodin; BA: Betulinic acid; BCP: β-caryophyllene; Ben: Benzyl acetone; ChA: Chlorogenic acid; Cos: Costunolide; DHL: Dehydrocostus lactone; IκBα: NF-κB inhibitor α; IL: Interleukin; IP: Inducible protein; LPS: Lipopolysaccharide; NK: Nootkatone; SD: Standard deviation; TNF-α: Tumor necrosis factor-α; THP-1: Human myeloid leukemia mononuclear cells; WCA: Wei Chang An pill.

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Similarly, our results showed that in LPS-induced THP-1 cells, Cos, BA, NK, BCP, DHL, and ChA significantly decreased the mRNA expression of pro-inflammatory cytokines, including *IP-10*, *TNF-α*, *IL-6*, and *IκBα*, whereas ATL and Ben had no obvious effects. Our data suggest that RA and LAR in WCA may play anti-inflammatory roles that help improve TNBS-induced UC in rats (Figure 3).

Rats with TNBS induction showed symptoms similar to those associated with patients with UC, including body weight loss, diarrhea, mucosal ulceration, and bloody stool, and thus are extensively used as acute UC models[31]. In our study, loss of body weight, increased DAI and CMDI scores, and shortened colon length (Figure 1A–D) were detected in UC rats. Furthermore, inflammatory cell infiltration in the colon

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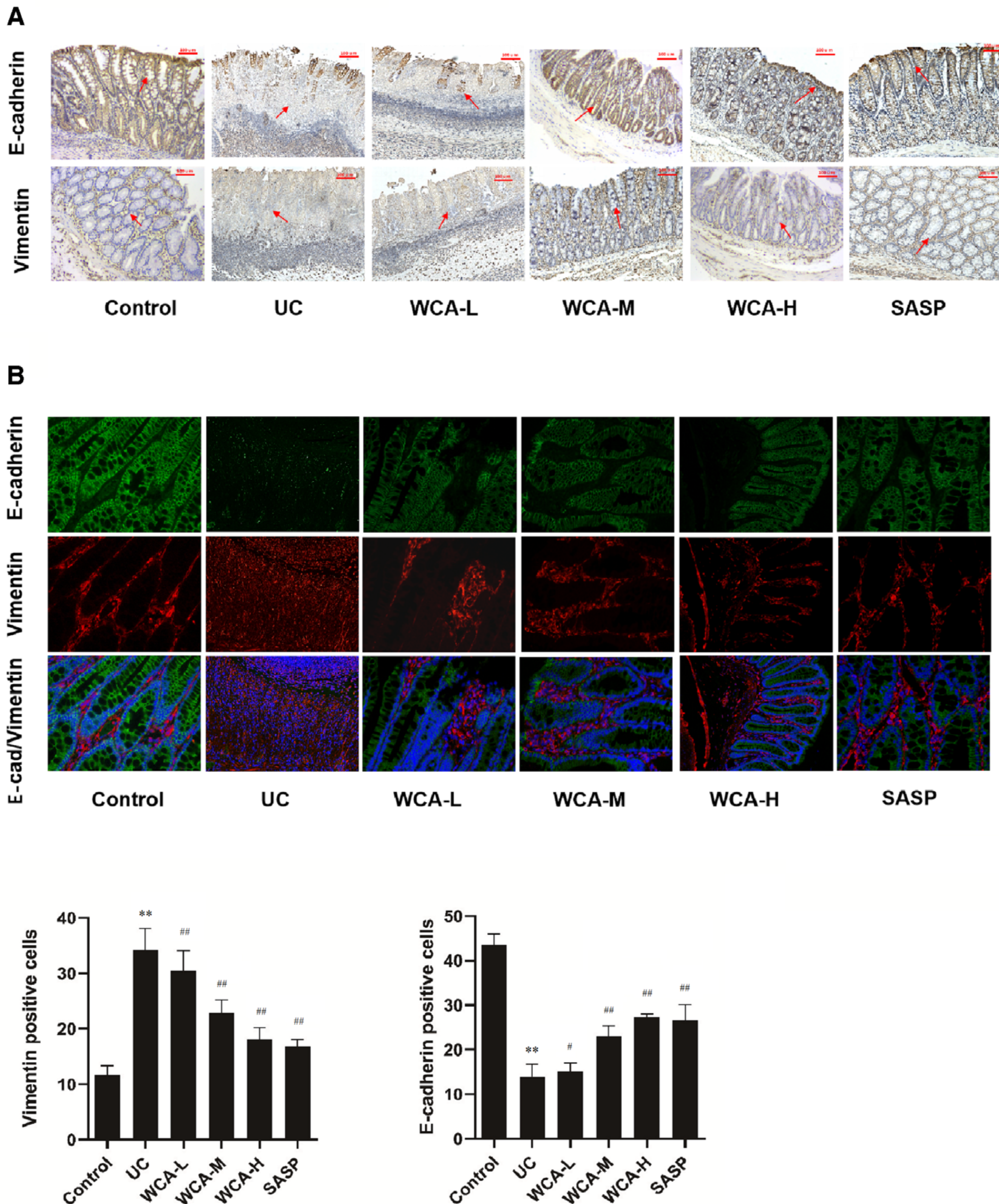

Figure 4. Effects of WCA on EMT-related protein expression. (A) Immunohistochemical staining. Images were taken at 100× magnification (scale bar: 100 μm). (B) Immunofluorescence double staining of E-cadherin and vimentin in rat colonic tissues. Images were taken at 40× magnification. Data are expressed as the mean ± SD (n = 6). ##P < 0.01 versus control group. *P < 0.05, **P < 0.01 versus UC group. EMT: Epithelial-mesenchymal transition; SASP: Sulfasalazine; SD: Standard deviation; UC, Ulcerative colitis; WCA: Wei Chang An pill.

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tissue (Figure 1E) and increased MPO activity in serum were also observed (Figure 1F) in the UC group, both of which are also typical and common symptoms in patients[32–33]. The mRNA expression of *TNF-α*, *IL-6*, *IL-18*, and *IL-1β* in colon tissues was highly upregulated in UC rats, which is similar to the results of previous studies[34–35]. In this study, TNBS-induced UC in rats was used to determine the effect of WCA on UC development (Figures 1, 2).

UC development is closely associated with exaggerated inflammation and the EMT process, which together contribute to the pathogenesis of UC based on clinical analysis and animal studies[36–38]. The transcriptomic analysis of colon tissues from 26 patients undergoing colectomy showed that genes related to EMT and inflammation are highly enriched[39]. In DSS-induced UC in mice, leukocyte infiltration of inflammatory tissue was found in colon epithelial cells, and the expression of E-cadherin was decreased[40]. Clinical biopsy studies have found that the upregulation of EMT markers occurs in active UC, which is considered a useful marker for cancer risk assessment in patients with UC[41]. It has also been shown that compared with the non-inflammatory proximal colonic mucosa, the DNA hypermethylation of EMT-related genes was observed in inflammatory colon samples, which aggravated the clinical phenotype of UC[9]. In this study, we established an inflammation-induced EMT process, both *in vitro* and *in vivo*. In UC rats, the downregulation of E-cadherin and the upregulation of vimentin were observed in TNBS-induced colon tissues (Figure 4), as reported in previous studies[40,42]. *In vitro* studies using Caco-2 cells showed that the inflammatory microenvironment was induced by incubation with medium from LPS-stimulated THP-1 cells. E-cadherin expression was downregulated and vimentin expression was upregulated in Caco-2 cells (Figure 5A, B), which provides a simple and convincing way to assess the cellular inflammation-related EMT process. Using these models, we further revealed that WCA not only suppressed UC-induced inflammatory cytokines but also inhibited inflammation-related EMT to improve UC symptoms.

Conclusions

Taken together, our findings demonstrate that WCA alleviates UC development by inhibiting the inflammation-related EMT process, highlighting the potential of WCA as a therapeutic agent for UC.

Conflicts of interest statement

The authors declare no conflict of interest.

Funding

This work was supported by the Science and Technology Project of Tianjin (22ZXGBSY00020 and 21ZYJFJC0070), National Key Research and Development Project of China (2021YFC1712904), and the National Natural Science Foundation of China (82074105). It was also supported by the Clinical Enhancement Project of traditional Chinese medicine from National Administration of Traditional Chinese Medicine.

Author contributions

Yaxin Qi, Meng Wang, and Lijuan Chai contributed equally to this study. Lin Miao and Peng Zhang initiated and designed the study; Yaxin Qi, Lijuan Chai, Sitong Jia, and Yi Wang completed the experimental research under the guidance and supervision of Lin Miao and Peng Zhang; Yaxin Qi and Lijuan Chai analyzed the data; Lin Miao, Yaxin Qi, and Min Zhang wrote the manuscript and drew the figures; Lin Wang, Yujing Wang, and Yaxin Qi assisted with statistical analysis; Han Zhang revised the manuscript. All authors have read and approved the final manuscript.

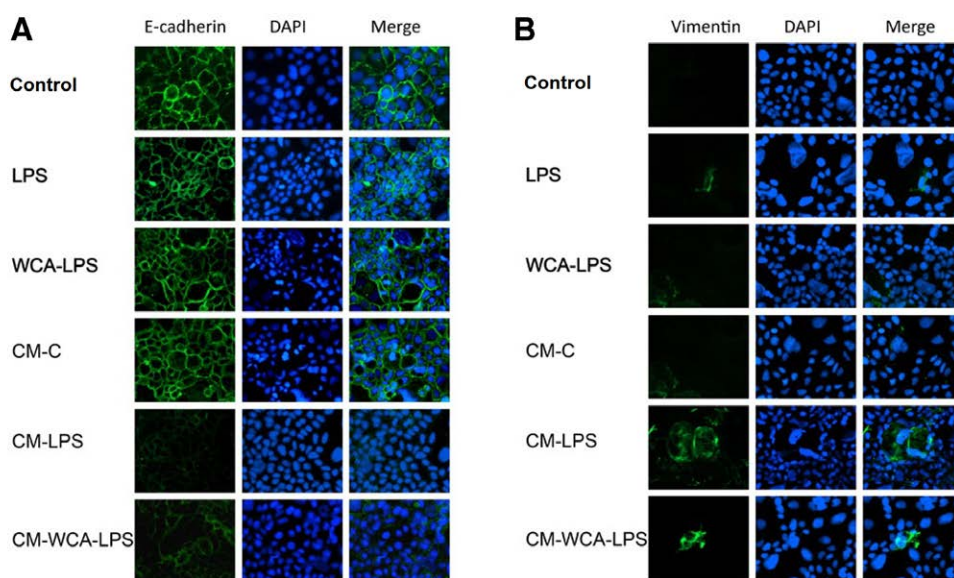

Figure 5. WCA-treated suppression of EMT *in vitro*. CM-WCA reversed the decrease of E-cadherin (A) and the increase of vimentin (B) in the Caco-2 cell treated with CM-LPS compared with the control (C, LPS, WCA-LPS, and CM-C) by immunofluorescent staining. Images were taken at 20× magnification (n = 6). CM: Conditioned medium; EMT: Epithelial-mesenchymal transition; LPS: Lipopolysaccharide; WCA: Wei Chang An pill.

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Ethical approval of studies and informed consent

The experimental procedures were approved by the Tianjin University of Traditional Chinese Medicine Animal Research Committee (TCM-LAEC20170018).

Acknowledgments

None.

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How to cite this article: Qi YX, Wang M, Chai LJ, Zhang M, Jia ST, Wichai N, Wang L, Wang YJ, Song JX, Zhang H, Wang Y, Zhang P, Miao L. Wei Chang An pill alleviates 2,4,6-trinitro-benzenesulfonic acid-induced ulcerative colitis by inhibiting epithelial-mesenchymal transition process. Acupunct Herb Med 2023;3(2):107–115. doi: 10.1097/HM9.0000000000000064