Research Paper

Experimental Study on the Treatment of Ulcerative Proctitis in Rats by Combining Baitouweng Decoction with Mesalamine Retention Enema

LI WEN FEI, T. DAN[1], LIU SHI HONG, LENG LI, CHEN MAO TING AND Y. Y. JUN*

Anorectal Department, Affiliated Hospital of North Sichuan Medical College, Sichuan, Nanchong 637001, [1]Anorectal Department, Suining First People's Hospital, Sichuan, Suining, Chuanshan 629000, China

To study the effect and mechanism of Baitouweng decoction, mesalamine and its combination with an enema in the treatment of ulcerative proctitis in rats is the objective of the study. The rats were divided into seven groups: Control (Normal), model (ulcerative proctitis), mesalamine, Baitouweng decoction, low-dose Baitouweng decoction combined with mesalamine, medium-dose Baitouweng decoction combined with mesalamine, high-dose Baitouweng decoction combined with mesalamine group. Except for the control group, all rats were induced with a 5 % acetic acid solution for ulcerative proctitis. The severity of ulcerative proctitis was measured by the disease activity index score; morphological changes of rectal tissue were observed by hematoxylin and eosin staining; expression of cytokines tumor necrosis factor alpha, interleukin-1 beta, interleukin-6, interferon-gamma, interleukin-10 messenger ribonucleic acid was detected by reverse transcription-polymerase chain reaction; Western blot analysis of signaling pathway proteins nuclear factor kappa B p65 and phosphorylated-nuclear factor kappa B p65 and the expression of tight junction proteins claudin-1, occludin and zonula occludens-1 was detected by Western blot and immunohistochemistry. The results revealed that the disease activity index scores of rats in each dosing group were significantly lower than those in the ulcerative proctitis group. After treatment, the expression of pro-inflammatory factors tumor necrosis factor alpha, interleukin-1 beta, interleukin-6 and interferon-gamma was inhibited and the expression of the anti-inflammatory factor interleukin-10 was up-regulated. In conclusion, Baitouweng decoction combined with mesalamine had a synergistic effect on the treatment of ulcerative proctitis. The mechanism of action may be to regulate the synthesis and release of inflammatory factors by inhibiting the over-activation of nuclear factor kappa B p65, reducing the damage of pro-inflammatory factors to rectal mucosa and repairing the intercellular tight junction structure, thus improving the intestinal barrier and reducing the rectal inflammatory response.

Key words: Baitouweng decoction, mesalamine, ulcerative proctitis, nuclear factor kappa B, inflammatory factors, tight junction proteins

Ulcerative Proctitis (UP) is a non-specific inflammatory disease of the intestine with lesions confined to the rectum. Its main pathological features are bleeding ulcers and inflammatory cell infiltration in the rectum. Studies have shown that the pathogenesis of UP may be related to genetic, environmental and immune dysfunction, intestinal epithelial barrier damage and dysbiosis[1-3]. However, the pathogenesis of UP is not clear, which is the main reason for the limited therapeutic options available. Baitouweng decoction is composed of *Pulsatilla chinensis* (Bunge) Regel (Baitouweng), *Phellodendron chinense* C.K. Schneid. (Huang Bai or Huang Bo), *Coptis chinensis* Franch (Huanglian) and *Fraxinus chinensis* Roxb (Qinpi). It has the effects of clearing heat and stopping dysentery, cooling the blood and detoxifying the toxin, which can treat a variety of intestinal diseases. Modern pharmacological research shows that Baitouweng decoction has antibacterial, anti-inflammatory, ulcer repair and immune regulating effects[4-6], but its mechanism of action on UP has not been clearly reported.

Nuclear Factor kappa B (NF-κB) is a widely expressed nuclear transcription factor and is considered to be one of the most important regulators

***Address for correspondence**
E-mail: yyjun2007@126.com

of the inflammatory process. When Nuclear Factor Inhibitor kappa B alpha (IκBα) is phosphorylated and degraded, the downstream NF-κB signaling pathway can be activated by p65 translocation into the nucleus, thus altering the expression of relevant inflammatory genes[7]. It has been shown that abnormal activation of the NF-κB signaling pathway causes downstream inflammatory factors such as Tumor Necrosis Factor alpha (TNF-α), Interleukin-(IL)-1 beta (β), IL-6, IL-10 and Interferon-gamma (IFN-γ) expression to be altered, causing a cascade reaction to disrupt the intestinal epithelial tight junction barrier, including claudin-1, occludin and Zonula Occludens-1 (ZO-1) and other tight junction proteins that increase mucosal permeability[8,9]. However, the effects and mechanisms of Baitouweng decoction and mesalamine on UP are still unclear. Based on the above theoretical basis, we hypothesized that the drugs might treat UP by inhibiting NF-κB activation[10].

MATERIALS AND METHODS

Animals and grouping:

56 healthy male Sprague-Dawley (SD) rats (204.30±5.98 g) aged 6-8 w was purchased from the Experimental Animal Center of North Sichuan Medical College, Animal Certificate No. SCXK (Chuan) 2018-18. Animals were randomly divided into 7 groups, each consisting of eight animals, including the UP group, the Mesalamine (MS) group (0.4 g/kg), the Baitouweng decoction (BTW) group (16 g/kg), the Low-dose Baitouweng decoction combined with Mesalamine (L-BTW+MS) group (BTW 8 g/kg+MS 0.4 g/kg), the Medium-dose Baitouweng decoction combined with Mesalamine (M-BTW+MS) group (BTW 16 g/kg+MS 0.4 g/kg), the High-dose Baitouweng decoction combined with Mesalamine (H-BTW+MS) group (BTW 32 g/kg+MS 0.4 g/kg) and the control group. UP was induced in rats by the administration of 5 % acetic acid as a preserved enema twice in 5 d. The rats in the experimental group were administered a retention enema for 10 d and the rats were executed by cervical dislocation after 10 d and rectal specimens were taken.

Preparation of Baitouweng decoction:

The Baitouweng decoction consisted of Baitouweng (15 g), Huang Bai (12 g), Qinpi (12 g) and Huanglian (6 g). All the herbs were purchased from the Chinese medicine pharmacy of the Affiliated Hospital of North Sichuan Medical College. The above drugs were steeped for 30 min at a concentration of 10:1 in water volume to drug weight ratio and decocted twice for 1 h each over a gentle fire. The decocted solution was filtered and the two filtrates were combined and concentrated to a suspension with mass concentrations of 0.8 g/ml, 1.6 g/ml and 3.2 g/ml (in terms of raw drug quantity) and stored at -20°.

Medicines and reagents:

Mesalamine enema (Lot no. 210283A) was obtained from Falk Pharmaceuticals in Germany; 5 % acetic acid solution (Lot no. 20220516) was obtained from Fuzhou Feijing Biotechnology Co., Ltd; the occult blood kit (Lot No. B220402) was obtained from Zhuhai Baso Biotechnology Co., Ltd; Hematoxylin and Eosin (HE) staining solution (Lot No. C0105S) was obtained from Shanghai Biyuntian Biotechnology Co., Ltd; Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) antibody (Lot No. AF7021) and claudin-1 antibody (Lot No. AF0127) were obtained from Affinity, United States of America (USA); NF-κB p65 antibody (Lot No. 10745-1-AP) was obtained from Proteintech, USA; phosphorylated (p)-NF-κB p65 antibody (Lot No. 3033) was obtained from Cell Signaling Technology, USA; occludin antibody (Lot No. GB111401) and ZO-1 antibody (Lot No. GB111402) was obtained from Wuhan Servicebio Co., Ltd; TNF-α, IL-1β, IL-6, IFN-γ and IL-10 primers were synched by Shanghai Sangon Biotech Co., Ltd.

Evaluation of UP severity based on the Disease Activity Index (DAI) score:

The DAI score has been widely used to evaluate the severity of UP in animal models. Briefly, an investigator complying with the protocol recorded and scored the changes in weight, hemoccult positivity or gross bleeding and stool consistency according to the previous report. The DAI score was a combination of scores for all these parameters mentioned. The scoring rules are shown in Table 1.

TABLE 1: SCORING CRITERIA OF DAI

Scoring	Weight loss rate (%)	Fecal trait	Fecal occult blood
0	0	Normal	Negative
1	1-5	Soft fecal	Fuchsia (1-2 min)
2	5-10	Mushy fecal	Fuchsia (10 s-1 min)
3	10-15	Watery fecal	Purple blue (10 s)
4	>15		Purple blue (Immediately)

Morphology and histological evaluation:

The rat rectal tissue was excised and fixed in 4 % paraformaldehyde. Then it was embedded in paraffin, sectioned and stained with HE. The extent of rectal mucosal injury was observed by microscopic photography.

Real-time Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR):

The total tissue Ribonucleic Acid (RNA) was extracted by the TRIzol one-step method by taking 20 mg of lyophilized rectal tissues and the RNA concentration and purity were detected by a K2800 nucleic acid analyzer. The RNA was then reverse transcribed into complementary Deoxyribonucleic Acid (cDNA), followed by conventional PCR amplification. Reaction system: 20 µl; reaction parameters: 95° preheat for 10 min, 40 cycles (95° denaturation for 10 s; 60° annealing for 30 s; fluorescence acquisition; 72° extension for 30 s). The gene primer sequences are shown in Table 2. The relative expression of the target gene messenger RNA (mRNA) was calculated using the $2^{-\Delta\Delta Ct}$ method with the standard housekeeping gene (GAPDH) as a reference.

Western blotting:

Rectal tissue was crushed in a mortar after the addition of liquid nitrogen, lysed and homogenized on ice with lysis solution, the supernatant was extracted by centrifugation, protein concentration was determined by the Bicinchoninic Acid (BCA) method, the membranes were transferred after instillation, closed and washed with Tris Buffered Saline+Tween 20 (TBST), add NF-κB p65, p-NF-κB p65, ZO-1, occludin and claudin-1 primary antibodies (1:1000) and incubate overnight at 4°. After rinsing the next day, add the secondary antibody (1:10 000) and incubate at room temperature for 1 h. After rinsing, add Enhanced Chemiluminescent (ECL) solution for color development and observations under the chemiluminescence imaging system.

Immunohistochemistry (IHC):

Paraffin sections of rectal tissue were dewaxed and rehydrated; microwave radiation antigen repair with citrate buffer was performed; 3 % hydrogen peroxide was blocked for 15 min; sheep serum blocking was performed; primary antibodies occludin (1:150), ZO-1 (1:200) and claudin-1 (1:200) were added dropwise and incubated overnight at 4°. Add secondary antibody, incubate at 37° for 30 min, add 3,3′-Diaminobenzidine (DAB) color developer, hematoxylin re-staining, dehydration and blocking. Under the microscope, cells with brownish-yellow granules between epithelial cells were observed as positive cells.

Statistical analysis:

All data were plotted and statistically analyzed using Prism 9.4.1 and Statistical Package for the Social Sciences (SPSS) 27.0 software. Data that conformed to a normal distribution with equal variance were compared between two groups using the t-test; multiple groups were compared using one-way Analysis of Variance (ANOVA); data with unequal variance were compared using the Welch test; data that did not conform to a normal distribution were compared between two groups using the Mann-Whitney test and multiple groups were compared using the Kruskal-Wallis test. Differences were considered statistically significant at $p<0.05$.

TABLE 2: PRIMER SEQUENCES OF TARGET GENES

Genes	Primer sequences (5'-3')	Length of product
GAPDH	ACAGCAACAGGGTGGTGGAC TTTGAGGGTGCAGCGAACTT	226 base pairs (bp)
TNF-α	ACAGCAACAGGGTGGTGGAC TTTGAGGGTGCAGCGAACTT	111 bp
IL-6	ACTTCCAGCCAGTTGCCTTCTTG TGGTCTGTTGTGGGTGGTATCCTC	110 bp
IL-1β	AATCTCACAGCAGCATCTCGACAAG TCCACGGGCAAGACATAGGTAGC	98 bp
IL-10	GGCAGTGGAGCAGGTGAAGAATG TGTCACGTAGGCTTCTATGCAGTTG	109 bp
IFN-γ	ACAACCCACAGATCCAGCACAAAG CACCGACTCCTTTTCCGCTTCC	100 bp

RESULTS AND DISCUSSION

Effects of Baitouweng decoction, mesalamine and their combination on clinical symptoms of acetic acid-induced proctitis in rats were shown in fig. 1A-fig. 1C. Rats in the control group exhibited normal activity, diet and fecal traits, as well as normal coat color and weight gain. However, compared with the control group, the model group showed reduced activity, dull yellow fur, bowed back and curled up, thin feces, mucus and blood stools, significantly decreased body weight and significantly higher DAI scores. In addition, as expected, the treated rats showed a significant reduction in DAI scores and the effect was most pronounced in the group with the combination of medium and high doses of Baitouweng decoction and mesalamine.

Histological observation and evaluation of each group of rats was shown here. HE staining showed that the normal group rats had intact rectal tissue structure, neatly arranged glands, more cup cells in the crypt, a closely spaced crypt, a regular surface of the crypt and no lesions were seen; the model group rats had severe defects in the rectal mucosal epithelium, disorganized and severely damaged glands, missing cup cells, a large infiltration of inflammatory cells, and multiple ulcer foci were seen; after treatment with drugs, the structure of the rectal mucosa was restored to different degrees, the crypt structure was intact, the number of cup cells was significantly increased, and inflammatory cells were significantly reduced compared with the model group. A large number of ulcer foci were also healed. The histopathological improvement of the rectum was more obvious in the combined drug group as shown in fig. 2.

Effects of Baitouweng decoction, mesalazine and their combination on cytokine mRNA expression in UP rats were shown in fig. 3A- fig. 3E. PCR results showed that the levels of pro-inflammatory factors TNF-α, IL-1β, IL-6 and IFN-γ in the rectal tissue of rats in the model group were higher than those in the control group but were significantly lower after treatment. However, the expression of the pro-inflammatory factor IL-10 mRNA was significantly lower in the UP group compared with the control group, whereas IL-10 mRNA expression was induced by drug treatment. Overall, the inhibition of inflammatory factor release was stronger in the group with the combination of medium and high doses of Baitouweng decoction and mesalamine.

Expression of NF-κB signaling pathway of genes and tight junction proteins was shown in fig. 4. Western Bolt analysis showed that the expression of nuclear factor receptor NF-κB p65 and its activated form, p-NF-κB p65, increased significantly in the rectal tissues of rats in the model group compared with the normal group and the expression of tight junction proteins ZO-1, occludin and claudin-1 decreased significantly. Compared with the model group, NF-κB p65 and p-NF-κB p65 protein expression levels were decreased to different degrees in each drug administration group, while ZO-1, occludin and claudin-1 protein expression levels were significantly increased, and the effect of the drug combination group was significantly better than that of the single-use group.

The IHC staining results showed that, compared with the control group, the epithelial cells in the model group were damaged and the yellow staining of the intercellular junctions was significantly less and lighter, suggesting a decrease in the expression of tight junction proteins; however, the yellow staining between the epithelial cells in all drug groups increased compared with the model group. Similarly, the combined drug group showed better results than the drug group alone as shown in fig. 5.

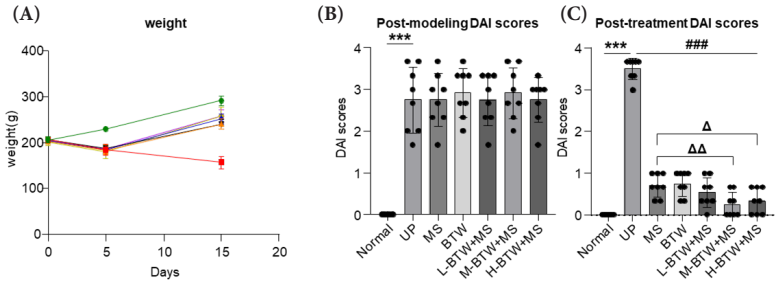

Fig. 1: Effects of Baitouweng decoction, mesalamine and their combination on clinical symptoms of acetic acid-induced proctitis in rats
Note: (A) Weight of rats in each group on d 0, d 5 and d 15, ( ) Control or Normal; ( ) UP; ( ) MS; ( ) BTW; ( ) L-BTW+MS; ( ) M-BTW+MS ( ) H-BTW+MS; (B) DAI scores of rats in each group after modeling and (C) DAI scores of rats in each group after treatment, *p<0.05, **p<0.01, ***p<0.001 *vs.* control group; #p<0.05, ##p<0.01, ###p<0.001 *vs.* model group and Δp<0.05, ΔΔp<0.01, ΔΔΔp<0.001 *vs.* mesalamine group

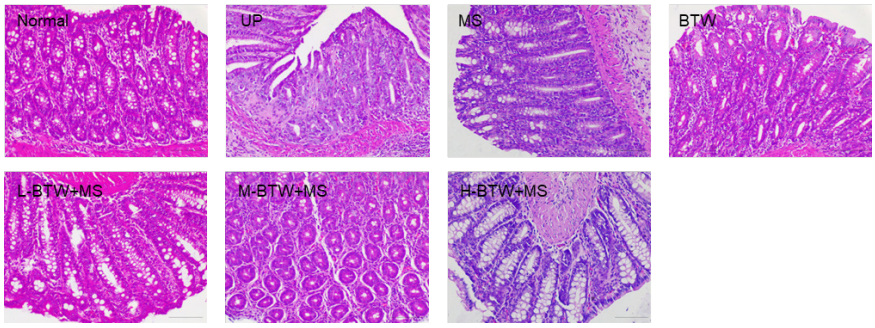

Fig. 2: Baitouweng decoction, mesalamine and their combination may reduce acetic acid-induced rectal inflammation (HE scale bar 200 µm)

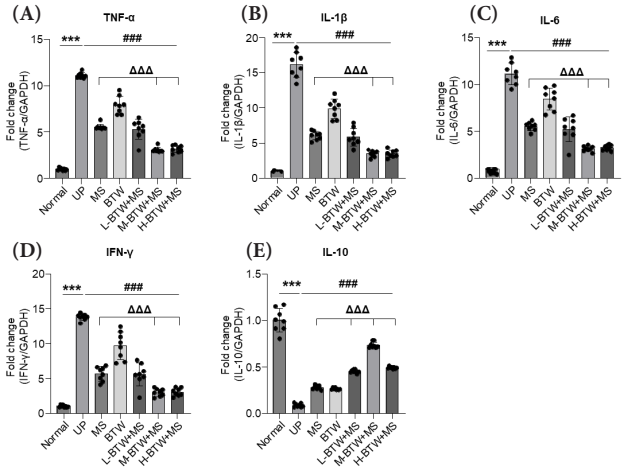

Fig. 3: Effects of Baitouweng decoction, mesalazine and their combination on cytokine mRNA expression in UP rats
Note: Expression of (A) TNF-α; (B) IL-1β; (C) IL-6; (D) IFN-γ and (E) IL-10 in UP rats, ***p<0.001 *vs.* control group; ###p<0.001 *vs.* model group and ΔΔΔp<0.001 *vs.* mesalamine group

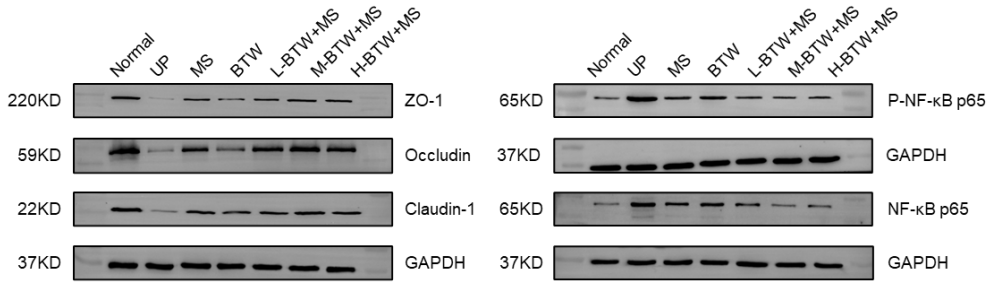

Fig. 4: Expression of NF-κB signaling pathway of genes and tight junction proteins

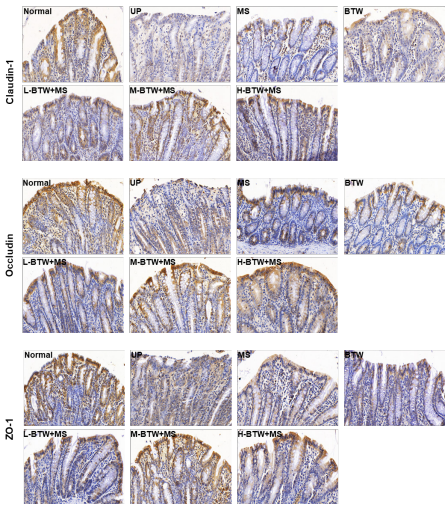

Fig. 5: The protein expressions of tight junction proteins (IHC scale bar 20 µm)

UP is a subtype of ulcerative colitis, the rectal type, which accounts for about 30 % of cases and the incidence of UP has increased to 55 % in the last decade[11,12]. If left untreated and allowed to develop, severe cases will involve the whole colon and even have the risk of cancer[13]. Therefore, the prevention and treatment of proctitis are essential. Although the exact triggers of UP are not known, the vicious cycle formed by the disruption of mucosal inflammation and epithelial barrier function and their interaction is the ultimate result[14]. Currently, the commonly used drugs in Western medicine for the treatment of UP include aminosalicylates, steroid hormones, biologics and Janus Kinase (JAK) inhibitors, among which aminosalicylates, represented by mesalamine, are the initial drugs of choice for treatment[15]. UP belongs to the category of "prolonged dysentery", or "diarrhea" in traditional Chinese medicine. The main pathogenesis of UP is stagnation of dampness and heat in the large intestine, blood and qi fighting against each other and stagnation of qi and blood, resulting in injury to blood channels[16]. Baitouweng decoction, as a representative prescription for the treatment of the syndrome of accumulated dampness heat of diarrhea in Chinese medicine, contains four herbs: Baitouweng, Huang Bo, Huanglian and Qinpi. The sovereign herb of Baitouweng decoction is Baitouweng, the minister herbs are Huanglian and Huang Bai and the assistant herb is Qinpi. The above four herbs work together to clear heat, act as an antitoxicant and cool blood for relieving dysentery. Modern research has shown that all four herbs have anti-inflammatory, anti-bacterial and anti-tumor effects[17-21]. In addition, the retention enema method has the advantages of preventing gastric juice from destroying medicine components, avoiding the first-pass effect of the liver and allowing the medicine to reach the lesion directly and be absorbed rapidly, which is often used in the treatment of the syndrome of accumulated dampness heat of enteritis. So in this experiment, we used Baitouweng decoction, mesalamine and the combination of the two drugs as retention enema in rats to investigate the mechanism of action and explore the effects of integrated traditional Chinese and Western medicine in the treatment of UP, with a view of providing a reference for the next treatment of UP as well as providing a new idea and potential target for its clinical treatment.

In this study, the UP rat's model was established by acetic acid enema method, the clinical signs of rats were observed and DAI scores were calculated. The results showed that the rats in the model group showed reduced activity, dark and yellow fur, weight loss, mucus-purulent blood stools, and significantly higher DAI scores; after dissection, the rectal mucosa was seen to be congested and edematous, and some of the recta adhered to the surrounding tissues; histological analysis showed that the rectal mucosal glands were severely damaged, with a large number of inflammatory cells infiltrating and multiple ulcer foci, indicating successful modeling. After 10 d of administration, the clinical symptoms of all groups of rats were improved, the DAI scores were significantly decreased, the mucosal damage was recovered and the effect of the combined group was more obvious than that of the mesalamine group. The effect was more obvious in the combination group than in the mesalamine group. It showed that both Baitouweng decoction and mesalamine could improve the symptoms of UP in rats, and the combined drug had a stronger effect on the improvement and repair of rectal mucosa in UP rats.

NF-κB p65 is a transcriptional regulator of inflammatory response, involved in inflammation, immune response and cell proliferation and differentiation processes, playing an important role in the regulation of inflammatory factors, adhesion factors and chemokines[22]. When NF-κB p65 is activated, it directly regulates the synthesis and release of inflammatory factors, among which the pro-inflammatory factors TNF-α, IL-1β, IL-6 and IFN-γ exacerbate intestinal mucosal tissue damage through immune upregulation and promotion of inflammatory activity[23]. The anti-inflammatory factor IL-10 functions to maintain tissue homeostasis during inflammation by limiting excessive inflammatory responses and promoting tissue repair mechanisms[24]. In the present study, we showed that both Baitouweng decoction and mesalamine can reduce intestinal inflammatory response by inhibiting NF-κB p65 protein activation, down-regulating pro-inflammatory factors and up-regulating the synthesis and release of anti-inflammatory factors through the NF-κB pathway, which is consistent with literature reports[25,26] and the modulating effect of the combination of medium and high doses of Baitouweng decoction and mesalamine was more significant in the group, suggesting that the combination of Baitouweng decoction and mesalamine does have a synergistic effect at certain drug concentrations.

Studies have found that some pro-inflammatory factors, such as TNF-α, IL-1β and IFN- γ can disrupt the intestinal epithelial tight junction barrier and increase intestinal mucosal permeability. While tight junction proteins mainly consist of the closure (claudin), occlusion (occludin) and band closure (ZO) families, occludin was the first identified tight junction transmembrane protein that binds to adjacent cells to produce paracellular closure, thereby controlling intercellular permeability; claudin-1 restricts ion entry into epithelial cells and interacts with other linker proteins in neighboring cells to form a fence that avoids lipid and protein diffusion and regulates the permeability of the tight junction complex and ZO-1 is mainly located at the boundary of adjacent epithelial cells and acts as an intracellular scaffolding protein that anchors occludin and claudin-1 to the cytoskeleton and synergistically controls the permeability of the intestinal barrier[27]. In the present study, we found that Baitouweng decoction combined with mesalamine could restore the tight junctional structure between cells by upregulating the expression of ZO-1, occludin and claudin-1 proteins and repairing the intestinal mucosal barrier, thereby treating UP.

In conclusion, Baitouweng decoction combined with mesalamine retention enema has a synergistic effect in UP treatment. The mechanism of action may be to regulate the synthesis and release of inflammatory factors by inhibiting the excessive activation of NF-κB p65, reducing the damage of pro-inflammatory factors to rectal mucosa and repairing the intercellular tight junction structure, thus improving the intestinal barrier and reducing the rectal inflammatory response.

Conflict of interests:

The authors declared no conflict of interest.

REFERENCES

1. Kaplan GG, Ng SC. Understanding and preventing the global increase of inflammatory bowel disease. Gastroenterology 2017;152(2):313-21.
2. Ray MK, Fenton CG, Paulssen RH. Novel long non-coding RNAs of relevance for ulcerative colitis pathogenesis. Noncoding RNA Res 2022;7(1):40-7.
3. Xuan-Qing CH, Xiang-Yu LV, Shi-Jia LI. Baitouweng decoction alleviates dextran sulfate sodium-induced ulcerative colitis by regulating intestinal microbiota and the IL-6/STAT3 signaling pathway. J Ethnopharmacol 2021;265:113357.
4. Wang YX, Ma KL, Wang Y, Wu DQ, Shao J, Wang TM, *et al.* Mechanism of butyl alcohol extract of Baitouweng Decoction (BAEB) on *Candida albicans* biofilms based on pH signal pathway. Zhongguo Zhong Yao Za Zhi 2019;44(2):350-6.
5. Miao Z, Chen L, Feng H, Gu M, Yan J, Xu Y, *et al.* Baitouweng decoction ameliorates ulcerative colitis in mice partially attributed to regulating Th17/Treg balance and restoring intestinal epithelial barrier. Front Pharmacol 2021;11:531117.
6. Wu Z, Yin B, Wang Z, Song E, You F. Clinical evidence and potential mechanisms in treating radiation enteritis with modified Baitouweng decoction. Evid Based Complement Alternat Med 2023;2023:1-27.
7. Zhu L, Gu P, Shen H. Protective effects of berberine hydrochloride on DSS-induced ulcerative colitis in rats. Int Immunopharmacol 2019;68:242-51.
8. Stahl M, Ries J, Vermeulen J, Yang H, Sham HP, Crowley SM, *et al.* A novel mouse model of *Campylobacter jejuni* gastroenteritis reveals key pro-inflammatory and tissue protective roles for toll-like receptor signaling during infection. PLoS Pathog 2014;10(7):e1004264.
9. Jimi E, Huang F, Nakatomi C. NF-κB signaling regulates physiological and pathological chondrogenesis. Int J Mol Sci 2019;20(24):6275.
10. Onkaramurthy M, Azeemuddin MM, Baig MR, Kendaganna PH, Rafiq M, Babu UV. Investigation of HPLF-111624 in modified experimental models of ulcerative proctitis and anal fissure in rats. J Exp Pharmacol 2022;14:149-65.
11. Ungar B, Kopylov U. Long-term outcome of ulcerative proctitis. United European Gastroenterol J 2020;8(8):847-8.
12. Wu XR, Liu XL, Katz S, Shen B. Pathogenesis, diagnosis and management of ulcerative proctitis, chronic radiation proctopathy and diversion proctitis. Inflamm Bowel Dis 2015;21(3):703-15.
13. D'Haens GR, Lindsay JO, Panaccione R, Schreiber S. Ulcerative colitis: Shifting sands. Drugs R D 2019;19(2):227-34.
14. Gecse KB, Lakatos PL. Ulcerative proctitis: An update on the pharmacotherapy and management. Expert Opin Pharmacother 2014;15(11):1565-73.
15. Burri E, Maillard MH, Schoepfer AM, Seibold F, van Assche G, Rivière P, *et al.* Treatment algorithm for mild and moderate-to-severe ulcerative colitis: An update. Digestion 2020;101(1):2-15.
16. Liu Y, Sun Y, Wang X, Wang D, Zeng L, Lu Q. Efficacy of the panax notoginseng ejiao suppository in the treatment of patients with ulcerative proctitis and its effect on inflammatory response and immune function. Dis Markers 2022;2022:1-7.
17. Dong Y, Qin Q, Zhong G, Mu Z, Cai Y, Wang X, *et al.* Integrated transcriptomic and metabolomic analyses revealed the molecular mechanism of terpenoid formation for salicylic acid resistance in *Pulsatilla chinensis* callus. Front Plant Sci 2022;13:1-18.
18. Yang Y, Qian C, Wu R, Wang R, Ou J, Liu S. Exploring the mechanism of the *Fructus mume* and *Rhizoma coptidis* herb pair intervention in ulcerative colitis from the perspective of inflammation and immunity based on systemic pharmacology. BMC Complement Med Ther 2023;23(1):1-4.
19. Li X, Cai K, Fan Z, Wang J, Wang L, Wang Q, *et al.* Dissection of transcriptome and metabolome insights into the isoquinoline alkaloid biosynthesis during stem development in *Phellodendron amurense* (Rupr.). Plant Sci 2022;325:111461.
20. Wang SK, Chen TX, Wang W, Xu LL, Zhang YQ, Jin Z, *et al.* Aesculetin exhibited anti-inflammatory activities through inhibiting NF-κB and MAPKs pathway *in vitro* and *in vivo*. J Ethnopharmacol 2022;296:115489.
21. Wang G, Lu M, Yao Y, Wang J, Li J. Esculetin exerts antitumor effect on human gastric cancer cells through IGF-1/PI3K/Akt signaling pathway. Eur J Pharmacol 2017;814:207-15.
22. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: Mechanism and clinical study. Signal Transduct Target Ther 2020;5(1):209.

23. Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, *et al.* Macrophage plasticity, polarization and function in health and disease. J Cell Physiol 2018;233(9):6425-40.
24. Ouyang W, O'Garra A. IL-10 family cytokines IL-10 and IL-22: From basic science to clinical translation. Immunity 2019;50(4):871-91.
25. Zhong Y, Zheng XB, Ye H, Guo M, Wu Q, Chi HG, *et al.* Efficacy and immune mechanism of Baitouweng tang on ulcerative colitis in rats. Chin J Exp Tradit Med Formul 2019:15-21.
26. Zhang D, Ma X, Wu H, Hong J, Zhang C, Wu L, *et al.* Efficacy of herb-partitioned moxibustion at Qihai (CV 6) and bilateral Tianshu (ST 25) on colonic damage and the TLR4/NF-κB signaling pathway in rats with Crohn's disease. J Tradit Chin Med 2018;38(2):218-26.
27. He Y, Huang XZ, Wei Z, Hao QL, Ding XW. Ameliorative effect and underlying mechanism of mulberry leaf alkaloids on D-galactose-induced intestinal barrier damage in mice. Food Sci 2022;43(7):139-46.

This article was originally published in a special issue, "Role of Biomedicine in Pharmaceutical Sciences" Indian J Pharm Sci 2023:85(2) Spl Issue "88-95"