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Clinical Study

Clinical efficacy of moxibustion for ulcerative colitis and its influence on vitamin D receptor

艾灸治疗溃疡性结肠炎临床疗效及对维生素D受体的影响

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Abstract

Objective: To observe the clinical efficacy of herbal cake-partitioned moxibustion for ulcerative colitis (UC) and elucidate its mechanism by targeting the vitamin D receptor (VDR) signaling pathway.

Methods: A total of 63 patients with UC were randomly divided into an observation group (30 cases, treated with herbal cake-partitioned moxibustion) and a control group (33 cases, treated with sham herbal cake-partitioned moxibustion). Moxibustion treatment was performed at Qihai (CV6) and bilateral Tianshu (ST25) and Shangjuxu (ST37), 3 times per week for 12 weeks. The total effective rate, visual analog scale (VAS) score for abdominal bloating and pain, and hospital anxiety and depression scale (HADS) score were compared between the two groups. Enzyme-linked immunosorbent assay was used to detect the concentrations of serum C-reactive protein (CRP), 25-hydroxyvitamin D [25(OH)D], and interleukin-12 (IL-12)/interleukin-23 (IL-23) p40. Immunohistochemistry was used to observe the expression levels of VDR and regenerating gene Ⅳ (Reg Ⅳ) proteins in colonic mucosa. The expression levels of VDR, cytochrome p450 27B1 (CYP27B1), and Reg Ⅳ mRNAs were detected by real-time fluorescence quantitive polymerase chain reaction.

Results: After treatment, the total effective rate in the observation group was 86.7%, which was significantly higher than 51.5% in the control group (P<0.05). After treatment, the VAS scores for abdominal bloating and pain in the observation group were significantly decreased (P<0.01), as well as the HADS-depression subscale (HADS-D) and HADS-anxiety subscale (HADS) scores (P<0.05), while only the VAS score for abdominal pain in the control group was reduced (P<0.05), and the improvements of the scores in the observation group were more significant than those in the control group (P<0.05). After treatment, the serum CRP concentrations in both groups and the IL-12/IL-23 p40 concentration in the observation group were significantly decreased (P<0.05), and the concentrations in the observation group were lower than those in the control group (P<0.05). The expression levels of VDR protein and mRNA in the colon in both groups were all increased (P<0.01), and the expression levels of Reg Ⅳ protein and mRNA and CYP27B1 mRNA were all decreased in the two groups (P<0.05 or P<0.01); the improvements in the observation group were more notable than those in the control group (P<0.05 or P<0.01).

Conclusion: Herbal cake-partitioned moxibustion can effectively alleviate abdominal pain and diarrhea in patients with UC, improve depression and anxiety disorders, and regulate the expression of related proteins in the VDR signaling pathway. The mechanism may be related to inhibiting intestinal inflammation by reducing the release of the proinflammatory cytokine IL-12/IL-23 p40.

Keywords: Moxibustion Therapy; Medicinal Cake-partitioned Moxibustion; Colitis, Ulcerative; Mucous Membrane; Vitamin D Receptor; Clinical Trial

【摘要】目的：观察隔药饼灸治疗溃疡性结肠炎(UC)的临床疗效，并从维生素D受体(VDR)信号通路角度阐释其效应机制。**方法：**将63例UC患者随机分为观察组30例(采用隔药饼灸治疗)和对照组33例(采用假隔药饼灸治疗)。两组均取气海和双侧天枢、上巨虚治疗，每周3次，共治疗12周。比较两组的总有效率、患者腹胀和腹痛视觉模拟量表(VAS)和医

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院焦虑抑郁量表(HADS)评分；酶联免疫吸附测定法检测血清C反应蛋白(CRP)、25羟维生素D[25(OH)D]、白细胞介素-12(IL-12)/白细胞介素-23(IL-23)p40的浓度；免疫组化法检测结肠VDR和再生基因蛋白Ⅳ(Reg Ⅳ)蛋白的表达；实时荧光定量聚合酶链反应检测结肠黏膜VDR、细胞色素P450家族成员27B1(CYP27B1)和Reg Ⅳ mRNA的表达。**结果：**治疗后，观察组总有效率为86.7%，明显高于对照组的51.5%(*P*<0.05)。治疗后，观察组腹痛和腹胀VAS评分降低(*P*<0.01)，HADS的抑郁亚量表(HADS-D)和焦虑亚量表(HADS-A)评分均降低(*P*<0.05)，对照组仅腹痛VAS评分明显降低(*P*<0.05)；观察组评分改善情况均优于对照组(*P*<0.05)。治疗后，两组患者血清CRP浓度、观察组血清IL-12/IL-23 p40浓度均降低(*P*<0.05)，观察组的浓度均低于对照组(*P*<0.05)；两组患者结肠VDR蛋白和mRNA的表达明显升高(*P*<0.01)，而Reg Ⅳ蛋白和mRNA以及CYP27B1 mRNA的表达均明显降低(*P*<0.05或*P*<0.01)，观察组较对照组改善更为显著(*P*<0.05或*P*<0.01)。**结论：**隔药饼灸能有效缓解UC患者腹痛、腹泻症状，改善抑郁、焦虑情绪，并能调节VDR信号通路相关蛋白的表达。其作用机制可能是通过减少促炎细胞因子IL-12/IL-23 p40的释放来抑制肠道炎症。

【关键词】灸法；药饼灸疗法；结肠炎，溃疡性；黏膜；维生素D受体；临床试验

【中图分类号】R245.8　　**【文献标志码】**A

Ulcerative colitis (UC) is a chronic intestinal nonspecific inflammatory disease involving the colonic mucosa, characterized by abdominal pain, increased defecation frequency, stool with mucous pus, and tenesmus[1]. In recent years, the incidence of UC in newly industrialized countries has gradually increased. A large-scale retrospective study showed that the overall incidence and prevalence of UC were 1.2-20.3 and 7.6-245 per 100 000 in one year, respectively, and has become the main disease of the digestive system[2-3]. The pathogenesis of UC is not fully understood but is thought to be mainly related to infection, genetic, immune, and psychological factors[4]. The role of vitamin D deficiency in inflammatory bowel disease (IBD) has been paid more and more attention[5], and the active metabolites of vitamin D can play a role by binding to the vitamin D receptor (VDR), which can regulate immunity and inhibit the expression of proinflammatory factors[6]. Antimicrobial peptides are a class of defensive peptide active substances against exogenous pathogens, which are highly expressed in colonic epithelial cells at the site of UC inflammation and play an important regulatory role in controlling intestinal inflammation[7]. Decreased VDR expression and abnormal expression of intestinal antimicrobial peptide regeneration gene Ⅳ (Reg Ⅳ) can cause UC colonic mucosal damage, which is one of the key links in the development of UC. At present, Western medicine mainly uses drugs such as aminosalicylic acid, glucocorticoids, and immunosuppressants for UC, but there are problems like poor clinical efficacy and significant side effects. Moxibustion has a good therapeutic effect on UC, and has the advantages of safety, low recurrence rate, and good long-term efficacy[8-10]. Our previous clinical studies have shown that moxibustion can effectively control intestinal inflammation, regulate intestinal mucosal immunity, and improve colonic mucosal damage in UC patients[11-15]. Therefore, in this randomized controlled trial, sham herbal cake-partitioned moxibustion was used as a control to elucidate the molecular mechanism of herbal cake-partitioned moxibustion for UC from the perspective of VDR signaling pathway.

1 Clinical Materials

1.1 Diagnostic criteria

The diagnostic criteria for UC referred to the *Consensus on the Diagnosis and Treatment of Inflammatory Bowel Disease (Guangzhou, 2012)*[16], in combination with a comprehensive evaluation of the clinical manifestations, colonoscopy, and colonic mucosal histological biopsy.

Key points of diagnosis: Suspected cases with typical clinical manifestations need further examination; patients with colonoscopic and/or radiographic features can be clinically diagnosed; the diagnosis can be confirmed in patients by histological features of colonic mucosa biopsy and/or surgical resection specimens; for those with the first onset, if the clinical manifestations, colonoscopy, and histological biopsy changes are not typical, UC cannot be diagnosed, but the cases should be followed up.

1.2 Inclusion criteria

Met the diagnostic criteria; aged 18-70 years old; not taking any medications other than mesalazine for UC symptoms; never received hormone therapy or received hormone therapy but limited to prednisone ≤0.5 mg/(kg·bw) daily; have not taken immune-suppressants or biological agents in the past 3 months; signed the informed consent form.

1.3 Exclusion criteria

Patients with heart, brain, liver, kidney, malignant tumors, and/or other diseases that seriously affect life activities; women during pregnancy or lactation; a family history of psychiatric or neurological genetic diseases; had poor adherence.

1.4 Statistical methods

The data were processed using the SPSS version 17.0 statistical software. The counting data were expressed as cases and percentages, and the Chi-square test was used for between-group comparisons. The

measurement data conforming to normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$), and the independent samples *t*-test was used for inter-group comparisons, and the paired *t*-test was used for the comparisons before and after treatment within the same group. Data that did not conform to normal distribution were expressed as median (minimum, maximum) [M (min, max)], with the Mann-Whitney *U* nonparametric test for inter-group comparisons and the Wilcoxon rank-sum test for intra-group comparisons before and after treatment. $P<0.05$ indicated statistical significance.

1.5 General data

This study was entrusted to the Ethics Committee of Shuguang Hospital, Shanghai University of Traditional Chinese Medicine (2016-487-38-01), filed with the Ethics Committee of Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, and registered at the National Institutes Clinical Trial Database (http://clinicaltrials.gov/, Registration No. NCT02931162). A total of 63 patients with UC were recruited from the Medical Outpatient Department of the Colitis Specialist Clinic in the Shanghai Research Institute of Acupuncture and Meridian. Thirty cases were randomly assigned to an observation group and 33 cases to a control group. There were no significant differences in gender, age, disease duration, disease stage, or other basic conditions between the two groups ($P>0.05$), indicating that the two groups were comparable (Table 1). Ten patients were randomly selected from the observation group and the control group, respectively, for serum and mucosal indicator detection.

Table 1 Comparison of the general data between the two groups

Group	*n*	Gender/case		Age/year [M (min, max)]	Disease duration/year [M (min, max)]	Disease stage/case	
		Male	Female			Mild	Moderate
Observation	30	13	17	48.5 (26.0, 70.0)	5.0 (1.0, 34.0)	24	6
Control	33	13	20	52.0 (25.0, 70.0)	4.8 (0.3, 30.0)	28	5

2 Treatment Methods

2.1 Observation group

The observation group received herbal cake-partitioned moxibustion at Qihai (CV6) and bilateral Tianshu (ST25) and Shangjuxu (ST37). The powders of *Fu Zi* (*Radix Aconiti Lateralis Praeparata*), *Dan Shen* (*Radix et Rhizoma Salviae Miltiorrhizae*), *Rou Gui* (*Cortex Cinnamomi*), *Mu Xiang* (*Radix Aucklandiae*), and *Hong Hua* (*Flos Carthami*) were mixed with yellow rice wine to make a thick paste, and a special mold was used to make herbal cakes of 2.3 cm in diameter and 0.5 cm in thickness. Cut pure moxa sticks (Nanyang Wolong Hanyi Wool Factory, China) into moxa cones of 17 mm in length and 18 mm in diameter (about 1.8 g). Place one ignited moxa cone on a herbal cake, and then put the herbal cake on each of the points to perform moxibustion. One moxa cone was used at each point for each treatment. The treatment was performed once every other day, 3 times a week, for 12 consecutive weeks.

2.2 Control group

The points, treatment frequency, and course of treatment in the control group were the same as those in the observation group, but a piece of cardboard wrapped in aluminum foil (2.3 cm in diameter and 0.2 cm in thickness) was cushioned between the herbal cake and the point during moxibustion to block the temperature, infrared radiation, and efficacy of the herbal cake during moxibustion, so as to suppress the effect of moxibustion to the greatest extent.

3 Efficacy Observation

3.1 Observed items

3.1.1 Visual analog scale (VAS)

VAS was used to assess the degree of abdominal bloating and pain. Drew a 10 cm long horizontal line on the paper, and one end of the horizontal line was marked 0, indicating no pain (no bloating) and scored 0 points; the other end was marked 10, indicating severe pain (severe bloating) and scored 10 points. Let the patient mark the corresponding position on the line according to their actual situation. A score of 0-3 points indicates mild; 4-7 points indicate moderate; 8-10 points indicate severe.

3.1.2 Emotional evaluation

The hospital anxiety and depression scale (HADS) was used to evaluate the mental and emotional state of patients[17].

3.1.3 Detection of the serum C-reaction protein (CRP), 25-hydroxyvitamin D [25(OH)D], and interleukin-12 (IL-12)/interleukin-23 (IL-23) p40 concentrations

A disposable BD vacuum non-anticoagulant blood collection tube of 5 mL was used to collect 2-3 mL of fasting venous blood of patients before and after treatment. After 30 min stay at room temperature, the sample was centrifuged at 4 ℃, 3 000 r/min, for 15 min. Then, pipette the light-yellow supernatant to the cryopreservation tube, and store it in a -80 ℃ refrigerator for later use. Automatic specific protein analyzer (DC-020, Aristo, China) and whole-blood CRP

antiserum (BA051, Shanghai Baidi Biotechnology Co., Ltd., China) were used to detect the serum CRP concentration before and after treatment in each group. According to the instructions, the human 25(OH)D enzyme-linked immunosorbent assay (ELISA) kit (ml038633-2, Shanghai Enzyme-linked Biotechnology Co., Ltd., China) and IL-12/IL-23 p40 ELISA kit (ml690280-2, Shanghai Enzyme-linked Biotechnology Co., Ltd., China) were used to detect the serum concentrations of 25(OH)D and IL-12/IL-23 p40 before and after treatment.

3.1.4 Detection of VDR and Reg Ⅳ protein expression in the colonic mucosa

Colonoscopy (painless anesthesia) was performed 1 week before treatment and 1 week after 12-week treatment, and colonic mucosal specimens were collected. Before treatment, colonoscopy was performed for mucous membrane at the lesion site in UC patients and its distance from the anus margin was recorded. After treatment, colonoscopy was performed for the mucosa at the same distance from the anus margin. Each patient contributed 2 pieces of colonic mucosa, one of which was stored in a -80 ℃ cryogenic freezer and the other in 10% neutral formalin fixative solution for later use.

The expression of VDR and Reg Ⅳ proteins in the colonic mucosa were detected by immune-histochemistry. After paraffin sections were dewaxed, inactivated by 3% H_2O_2 and repaired by antigen, 5% BSA sealing solution was added. Added an appropriate amount of diluted VDR (ab3508, Abcam, UK) and Reg Ⅳ primary antibody (AB89917, Abcam, UK) dropwise, overnight at 4 ℃. Then the secondary antibody was added dropwise, hematoxylin counterstained for 2 min, 1% hydrochloric acid alcohol differentiated for 5 s, 2% ammonia turned blue for 20 s, and then neutralized gum sealed after dehydration with absolute ethanol and xylene, and observed under light microscope. Brownish-yellow indicated positively expressed. Selected 3 non-overlapping fields of view per slice. Image-Pro Plus version 6.0 image analysis software was used to analyze and measure the integrated optical density (IOD).

3.1.5 Detection of colonic mucosal VDR, cytochrome p450 27B1 (CYP27B1) and Reg Ⅳ mRNAs

The expression of VDR, CYP27B1, and Reg Ⅳ mRNAs in the colonic mucosa was detected by real-time fluorescence quantitive polymerase chain reaction (RT-qPCR). The total RNA of the colon was extracted by trizol (Invitrogen, USA), and the Nanodrop spectrophotometer (Thermo Electron Corporation, USA) detected the quality and quantity of RNA. Reverse transcription-polymerase chain reaction kit (RR0371A, Takara, Japan) was used for reverse transcription. The sequences were obtained from the target genes in Gene Bank database. The primers were designed by NCBI Primer-blast and synthesized by Suzhou Jinweizhi Biotechnology Co., Ltd., China, as shown in Table 2. The reverse transcription product was amplified on a real-time polymerase chain reaction (PCR) system (Roche 480Ⅱ, Switzerland) using a PCR kit (208052, QuantiNova SYBR Green PCR kit, QIAGEN, Germany) under reaction conditions of 95 ℃ for 2 min, 95 ℃ for 5 s, 60 ℃ for 10 s, for a total of 50 cycles.

Table 2 Primers for mRNA detection

Name	Amplification length/bp	Upstream primer sequence (5'→3')	Downstream primer sequence (5'→3')
VDR	149	CCAGTTCGTGTGAATGATGG	AGATTGGAGAAGCTGGACGA
CYP27B1	115	TGTTTGCATTTGCTCAGAGG	AACAGGAAGTGGGTCAGGTG
Reg IV	187	TGCTCCTGGATGGTTTTACC	TATCGGCTGGCTTCTCTGAT
β-actin	136	GCAGAAGGAGATCACTGCCCT	GCTGATCCACATCTGCTGGAA

Note: VDR=Vitamin D receptor; CYP27B1=Cytochrome p450 27B1; Reg IV=Regenerating gene IV.

3.2 Efficacy criteria

The total effective rate was used as the main efficacy indicator, and the evaluation criteria referred to the clinical efficacy evaluation standard for UC in the *Diagnosis and Treatment of Inflammatory Bowel Disease (Guangzhou, 2012)*[16].

Remission: Clinical symptoms disappeared, colonoscopy showed mucosa generally normal or no active inflammation.

Effective: Clinical symptoms basically disappeared; colonoscopy showed mild inflammation of the mucosa.

Invalid: There was no improvement in clinical symptoms or colonoscopy.

3.3 Results

3.3.1 Comparison of the clinical efficacy

After treatment, the total effective rate of the control group was 51.5% and that of the observation group was 86.7%, and the difference between the two groups was statistically significant (P<0.05), suggesting that the total effective rate of herbal cake-partitioned moxibustion for UC is better than that of sham herbal cake-partitioned moxibustion (Table 3).

3.3.2 Comparison of the VAS scores for abdominal pain and bloating

Before treatment, there were no significant

differences in the VAS scores between the two groups for abdominal pain and bloating (*P*>0.05). After treatment, the VAS scores for abdominal pain and bloating in both groups were reduced (*P*<0.01, *P*<0.05), and the scores of the observation group were lower than those in the control group (*P*<0.05, *P*<0.01). See Table 4.

3.3.3 Comparison of the mental and emotional scores

Before treatment, there were no significant differences in the scores of the HADS-depression subscale (HADS-D) and HADS-anxiety subscale (HADS-A) between the two groups (*P*>0.05). After treatment, the HADS-D and HADS-A scores in the observation group decreased (*P*<0.01, *P*<0.05), and there was no significant change in either score in the control group (*P*>0.05). The changes in the HADS-D (*P*<0.01) and HADS-A (*P*<0.05) scores after treatment in the observation group were greater than those in the control group (Table 5 and Table 6).

Table 3 Comparison of the clinical efficacy between the two groups **Unit: case**

Group	*n*	Remission	Effective	Invalid	Total effective rate (%)	χ^2-value	*P*-value
Observation	30	12	14	4	86.7	9.14	0.01
Control	33	9	8	16	51.5		

Table 4 Comparison of the VAS scores for abdominal bloating and pain between the two groups [M (min, max)] **Unit: point**

Group	*n*	VAS score for abdominal pain				VAS score for abdominal bloating			
		Pre-treatment	Post-treatment	*Z*-value	*P*-value	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	30	3 (1, 8)	1 (0, 3)	-3.861	0.004	3 (0, 8)	1 (0, 4)	3.312	0.001
Control	33	3 (0, 8)	2 (0, 5)	-2.033	0.042	3 (0, 7)	3 (0, 5)	-1.027	0.304
Z-value		-0.702	-1.983			-0.448	-4.112		
P-value		0.483	0.047			0.654	<0.001		

Note: VAS=Visual analog scale.

Table 5 Comparison of the HADS-D score between the two groups [M (min, max)] **Unit: point**

Group	*n*	Pre-treatment	Post-treatment	Difference value	*Z*-value	*P*-value
Observation	30	4.0 (0, 13.0)	2.5 (0, 11.0)	1 (-4, 5)	-1.950	0.001
Control	33	3.0 (0, 10.0)	3.0 (0, 11.0)	-1 (-4, 2)	-2.594	0.784
Z-value		-0.306	-2.098	-2.594		
P-value		0.759	0.036	0.009		

Note: HADS-D=Hospital anxiety and depression scale-depression subscale.

Table 6 Comparison of the HADS-A score between the two groups [M (min, max)] **Unit: point**

Group	*n*	Pre-treatment	Post-treatment	Difference value	*t*-value	*P*-value
Observation	30	5.43±3.00	4.63±2.98	0 (-7, 5)	-2.178	0.029
Control	33	5.85±3.56	6.12±2.98	0 (-7, 2)	-3.145	0.268
Statistical value		0.497[1)]	1.981[1)]	-2.178[2)]		
P-value		0.621	0.052	0.029		

Note: HADS-A=Hospital anxiety and depression scale-anxiety subscale; 1) *t*-value; 2) *Z*-value.

3.3.4 Comparison of the serum CRP, 25(OH)D, and IL-12/ IL-23 p40 concentrations

There were no significant differences in the serum CRP, 25(OH)D, and IL-12/IL-23 p40 concentrations between the two groups before treatment (*P*>0.05). After treatment, the serum CRP concentrations of patients in both groups decreased significantly (*P*<0.05), the serum IL-12/IL-23 p40 concentration in the observation group also decreased (*P*<0.05), and the serum CRP and IL-12/IL-23 p40 concentrations in the observation group were lower than those in the control group (*P*<0.05), but there was no significant difference in the concentration of 25(OH)D after treatment between the two groups (*P*>0.05). See Table 7-Table 9.

3.3.5 Comparison of the VDR and Reg Ⅳ protein expression in colonic mucosa

VDR was mainly expressed in the cytoplasm and on the nucleus. There was no significant difference in the

expression of VDR protein in the colonic mucosa between the two groups before treatment (P>0.05), indicating the comparability. After treatment, the expression levels of VDR protein in the colonic mucosa in both groups were significantly increased (P<0.01), and the expression level of VDR protein in the observation group was higher than that in the control group (P<0.05). See Figure 1 and Table 10.

Table 7 Comparison of the serum CRP between the two groups [M (min, max)] **Unit: mg/L**

Group	*n*	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	10	1.83 (0.50, 5.30)	0.50 (0.50, 0.90)	-1.847	0.021
Control	10	1.55 (0.50, 5.16)	0.71 (0.50, 1.20)	-3.484	0.015
Z-value		-0.306	-2.098		
P-value		0.759	0.036		

Note: CRP=C-reactive protein.

Table 8 Comparison of the serum 25(OH)D between the two groups [M (min, max)] **Unit: mg/L**

Group	*n*	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	10	8.39 (0.76, 47.10)	11.14 (1.37, 27.21)	-0.428	0.817
Control	10	11.29 (0.29, 43.51)	10.16 (0.96, 36.55)	-0.246	0.749
Z-value		-0.227	-0.302		
P-value		0.821	0.762		

Note: 25(OH)D=25-hydroxyvitamin D.

Table 9 Comparison of the serum IL-12/IL-23 p40 between the two groups [M (min, max)] **Unit: mg/L**

Group	*n*	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	10	17.63 (7.08, 121.76)	15.45 (1.15, 27.18)	-1.178	0.001
Control	10	43.60 (20.46, 159.62)	31.91 (1.13, 45.78)	-1.883	0.178
Z-value		-1.739	2.439		
P-value		0.082	0.025		

Note: IL-12/IL-23=Interleukin-12/interleukin-23.

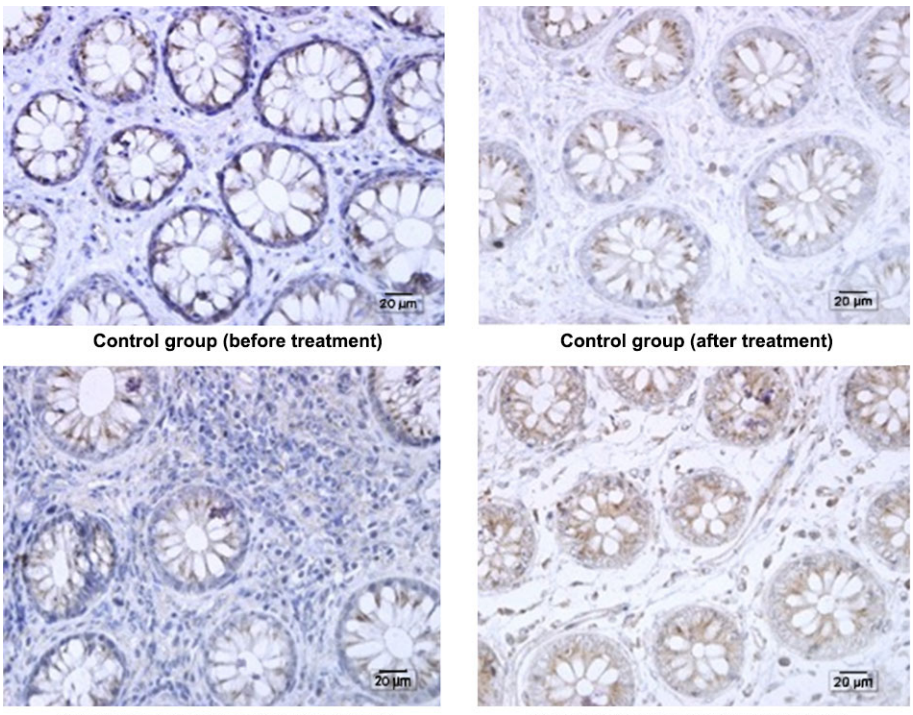

Figure 1 Expression of VDR protein in colonic mucosa in the two groups (immunohistochemistry, × 400)

Table 10 Comparison of the IOD value of VDR protein in colonic mucosa between the two groups [M (min, max)]

Group	*n*	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	10	132 473.91 (18 856.75, 400 226.63)	587 346.02 (308 586.19, 1 483 109.10)	-1.803	0.002
Control	10	89 379.52 (10 657.21, 507 843.00)	264 905.58 (100 801.00, 845 054.38)	-2.457	0.001
Z-value		-0.529	-2.220		
P-value		0.597	0.040		

Note: IOD=Integrated optical density; VDR=Vitamin D receptor.

Reg Ⅳ was mainly expressed in the cytoplasm. There was no significant difference in the expression of Reg Ⅳ protein in the colonic mucosa between the two groups of patients before treatment (P>0.05), suggesting comparability. After treatment, the expression of Reg Ⅳ protein in the colonic mucosa of both groups was significantly reduced (P<0.05, P<0.01), and the expression level of Reg Ⅳ protein in the observation group was lower than that in the control group (P<0.05). See Figure 2 and Table 11.

3.3.6 Comparison of the VDR, CYP27B1, and Reg Ⅳ mRNAs expression in colonic mucosa

Before treatment, there were no significant differences in the expression levels of VDR, CYP27B1, and Reg Ⅳ mRNAs in the colonic mucosa between the two groups (P>0.05). After treatment, the expression levels of VDR mRNA in the colonic mucosa in both groups increased significantly (P<0.01), and the expression level of VDR mRNA in the observation group was higher than that in the control group (P<0.05). The expression levels of CYP27B1 mRNA and Reg Ⅳ mRNA in the colonic mucosa in the two groups were significantly reduced (P<0.01, P<0.05), and the expression levels of CYP27B1 mRNA and Reg Ⅳ mRNA in the observation group were lower than those in the control group (P<0.01, P<0.05). See Table 12-Table 14.

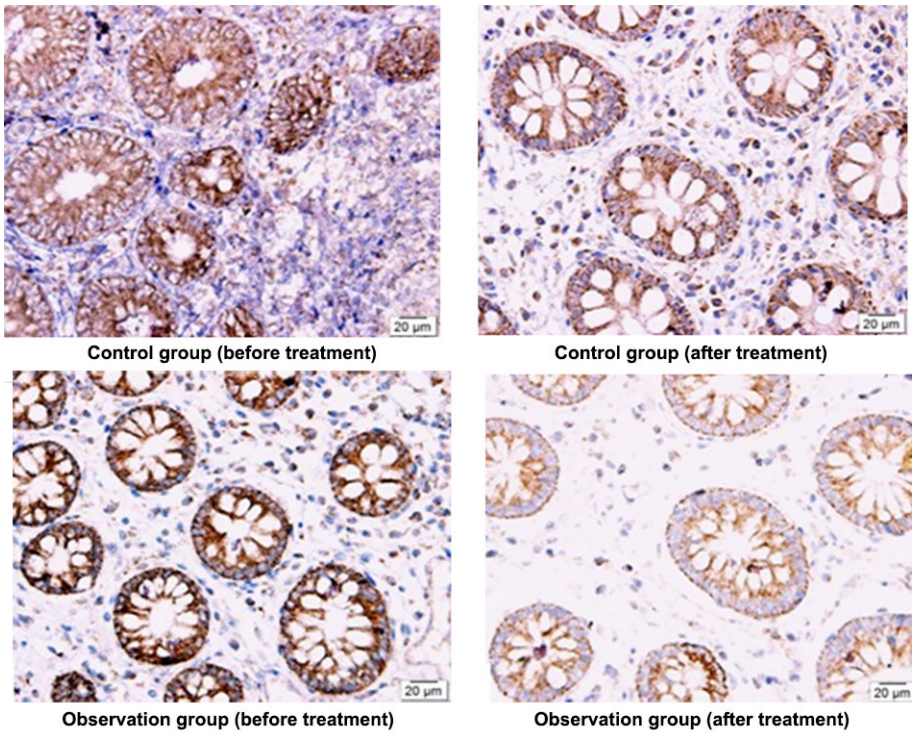

Figure 2 Expression of Reg IV protein in colonic mucosa in the two groups (immunohistochemistry, × 400)

Table 11 Comparison of the IOD value of Reg IV protein in colonic mucosa between the two groups [M (min, max)]

Group	*n*	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	10	405 156.38 (144 171.83, 1 042 094.30)	110 939.66 (12 543.17, 297 496.31)	-3.158	0.001
Control	10	454 498.93 (104 125.68, 954 937.25)	229 604.20 (85 076.09, 454 937.25)	-0.895	0.035
Z-value		-0.454	2.162		
P-value		0.650	0.044		

Note: IOD=Integrated optical density; Reg IV=Regenerating gene IV.

Table 12 Comparison of the VDR mRNA in colonic mucosa between the two groups [M (min, max)]

Group	*n*	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	10	1.45 (0.70, 1.82)	6.73 (1.53, 13.55)	-2.803	0.005
Control	10	1.01 (0.44, 2.22)	2.17 (1.75, 4.76)	-2.599	0.009
Z-value		-1.042	-2.080		
P-value		0.311	0.038		

Note: VDR=Vitamin D receptor.

Table 13 Comparison of the Reg IV mRNA in colonic mucosa between the two groups [M (min, max)]

Group	*n*	Pre-treatment	Post-treatment	*Z*-value	*P*-value
Observation	10	5.88 (0.77, 9.92)	0.78 (1.40, 4.99)	-1.988	0.047
Control	10	2.99 (0.81, 12.55)	1.04 (0.84, 4.79)	-2.701	0.026
Z-value		-0.092	-2.042		
P-value		0.928	0.041		

Note: Reg IV=Regenerating gene IV.

Table 14 Comparison of the CYP27B1 mRNA in colonic mucosa between the two groups ($\bar{x}$ ±*s*)

Group	*n*	Pre-treatment	Post-treatment	*t*-value	*P*-value
Observation	10	4.49±2.61	0.64±0.30	-2.803	0.001
Control	10	5.35±1.78	1.13±0.25	-2.105	0.005
t-value		0.863	4.019		
P-value		0.400	0.001		

Note: CYP27B1=Cytochrome p450 27B1.

4 Discussion

UC belongs to the categories in traditional Chinese medicine such as “diarrhea”, “dysentery”, “stagnant diarrhea”, “intestinal afflux”, etc., and the disease is located in the intestine, and closely related to the spleen, liver, and kidney. Moxibustion therapy is an important part of acupuncture-moxibustion therapy, with the effects of warming the meridians and dissipating cold, tonifying the deficiency and promoting Yang, and unblocking the meridians and collaterals, and can be used to treat a variety of diseases. Moxibustion therapy has been widely used to treat digestive diseases and has good clinical efficacy[18-22]. Our team has been treating UC with herbal cake-partitioned moxibustion for a long time. The herbal cake is mainly composed of *Fu Zi*, *Dan Shen*, *Rou Gui*, *Mu Xiang*, and *Hong Hua*. It has the function of warming Yang, strengthening the spleen, and regulating Qi and blood, and can effectively improve the clinical symptoms and quality of life of UC patients[14-15]. In this study, moxibustion at bilateral Tianshu (ST25) and Shangjuxu (ST37), and Qihai (CV6). Tianshu (ST25) is the Front-Mu Point of the large intestine; Shangjuxu (ST37) is the Lower He-Sea Point of the large intestine. According to the theories that “Front-Mu Point is used to treat diseases of Fu-organs”, “Lower He-Sea Point is used to treat internal Fu-organs”, and “combination of He-Sea Point and Front-Mu Point", the two points are often used to treat colorectal diseases, and play the role of modulating the intestines, clearing heat and dampness, anti-diarrhea, and analgesia. Qihai (CV6) has the effect of benefiting Qi and Yang, and tonifying Yuan-Primordial Qi and deficiency. These three points are commonly used in acupuncture-moxibustion treatment of UC[23-24]. The results of this study showed that the scores of VAS for abdominal bloating and pain and serum CRP concentration in UC patients treated with herbal cake-partitioned moxibustion were significantly reduced, and they were significantly lower than those in patients treated with sham herbal cake-partitioned moxibustion.

Studies have shown that the disease progression in approximately 74% of UC patients is related to psychosocial factors[25-26]. Clinical observation has found that UC patients have a variety of mental health problems, with different degrees of anxiety and depression being the most common, and the incidence is high[27-29]. Many UC patients have significant anxiety or depression due to long-term failure to relieve symptoms, and this stress or mood, in turn, affects gastrointestinal function. Psychosocial and stress stimulation can act on the central nervous system and change the sensory, motor, secretion, and other functions of the gastrointestinal tract through the brain-

intestinal axis pathway, resulting in gastrointestinal dysfunction and the occurrence of corresponding diseases. This study showed that compared with sham herbal cake-partitioned moxibustion, herbal cake-partitioned moxibustion can significantly reduce the mood scale score of UC patients and relieve their depression and anxiety symptoms. However, whether herbal cake-partitioned moxibustion improves abnormal emotional activity by repairing colon injury in UC patients, or reduces the malignant feedback of abnormal mental emotions to UC lesions by improving abnormal brain responses needs further research.

Vitamin D deficiency was strongly associated with colitis[30]. Vitamin D deficiency has been found in approximately 45% of UC patients[31]. Patients with UC often have gastrointestinal disorders and lipid absorption, which can lead to insufficient vitamin D intake[32], and vitamin D deficiency can further aggravate UC, and the two interact and are mutually causal[33]. The main active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2$-D_3), has a wide range of effects *in vivo*, mainly playing a role by binding to VDR. In addition to regulating calcium and phosphorus metabolism, it can also inhibit proliferation, promote differentiation, regulate immunity, inhibit cell necrosis, and suppress tumor infiltration and metastasis, alongside other non-calcium-modulatory effects. In a state of vitamin D deficiency, VDR can be activated by agonists to suppress the inflammatory response to colitis in UC patients[34-36]. In addition, Th17 cells, a subset of $CD4^+$ T lymphocytes, release IL-12/IL-23 p40, causing colonic tissue damage[37]. Experimental studies have found that the neutralizing agent of IL-12/IL-23 p40 can be effective in treating IBD[38-39]. The results showed that after 12 weeks of treatment, herbal cake-partitioned moxibustion significantly reduced the serum IL-12/IL-23 p40 concentration in UC patients and inhibited inflammatory responses. Secondly, the serum 25(OH)D concentrations in both groups were less than 10 ng/mL, indicating that these patients had severe vitamin D deficiency. However, there was no intra-group or inter-group statistically significant difference in serum 25(OH)D after treatment, which may be related to the complexity of the causes of vitamin D deficiency in UC patients, such as increased disease activity, inflammation of the colonic mucosa, insufficient sunshine hours, insufficient intake, smoking, and/or lower gastrointestinal resection. In addition, although numerous studies have shown a strong relationship between vitamin D and the occurrence of UC[40-41], there are reports that do not support this view[42]. Therefore, whether herbal cake-partitioned moxibustion can regulate the concentration of serum 25(OH)D in UC patients still needs further research by expanding the intervention time or dose.

CYP27B1 is an intermediate required for vitamin D metabolism to produce 1,25-$(OH)_2$-D_3. At a low concentration of 1,25-$(OH)_2$-D_3, VDR agonists can induce the primary response genes of VDR cytochrome P450 family 2 subfamily R member 1, CYP27B1, cytochrome P450 family 24 subfamily A member 1 and antimicrobial peptides, and their anti-inflammatory effect is significantly better compared with 1,25-$(OH)_2$-D_3[34-36], indicating that vitamin D supplementation may not be effective in UC patients. However, direct stimulation of VDR receptors, initiation of its primary response genes, and then cascade reaction can effectively control the release of inflammatory cytokines from the colon and control colonic inflammation. After knocking out the VDR gene, the degree of colitis induced by dextran sulfate sodium salt can be aggravated, and the expression of UC colonic epithelial VDR can be significantly reduced. Transfection of human VDR can target the intervention of $VDR^{-/-}$ knockout intestinal epithelial cells of mice to reduce colitis, that is, the expression level of VDR can directly affect the degree of colitis in UC mice[43]. In addition, Reg Ⅳ is closely related to intestinal mucosal damage; when the colon is infected, the body can increase the expression of mucin to resist the invasion of intestinal pathogenic microorganisms. The serum CRP level in UC patients is positively correlated with the increased expression of colonic Reg Ⅳ[44]. *In vitro* studies have found that the strong expression of Reg Ⅰα and Reg Ⅳ occurs in colonic epithelial cells with inflammation in UC, and the expression level of Reg Ⅳ in UC tissue was affected by the expression of basic fibroblast growth factor and hepatocyte growth factor[45].

In this study, after 12 weeks of treatment, compared with the pre-treatment levels, the expression levels of CYP27B1 mRNA and Reg Ⅳ mRNA and its protein in the colonic mucosa of the observation group were significantly reduced, and the expression levels of VDR mRNA and its protein were significantly increased, and the differences between the observation group and the control group were statistically significant. Therefore, we believe that herbal cake-partitioned moxibustion may achieve the purpose of treating UC by reducing the expression of CYP27B1 mRNA in the colonic mucosal tissue of UC patients, increasing the expression of VDR mRNA and its protein, reducing the concentration of Reg Ⅳ mRNA and its protein and serum IL-12/IL-23 p40, and inhibiting inflammatory responses.

In summary, herbal cake-partitioned moxibustion can effectively treat UC, and one of its mechanisms may be the regulation of VDR signaling pathway. However, there are some shortcomings in this study, such as the small number of clinical cases included, which may have a certain impact on the final level of the observed indicators. In addition, this study is an initial exploration of the immunomodulatory mechanism of herbal

cake-partitioned moxibustion for UC from the perspective of VDR signaling pathway. Due to limited sample size and depth, the later research stage will also be combined with animal experiments to deeply explore the mechanism of action of herbal cake-partitioned moxibustion for UC at the genetic level and by targeting intestinal flora.

Conflict of Interest

Author WU Huangan is editor-in-chief of the *Journal of Acupuncture and Tuina Science*. The paper was handled by other editors and has undergone a rigorous peer review process. Author WU Huangan was not involved in the journal's review or decisions related to this manuscript.

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Statement of Informed Consent

Informed consent was obtained from all individual participants.

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