

Original Research Article

Therapeutic efficacy of a combination of mesalazine and Bifid Triple Viable Capsules (BTVCs) on ulcerative colitis patients, and its effect on inflammation and oxidative stress

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Abstract

Purpose: To determine the curative impact of mesalazine (MSZ)-BTVCs combination on ulcerative colitis (UC), and its influence on inflammation and oxidative stress in the patients.

Methods: 100 UC patients were randomly assigned to a control group given MSZ capsule treatment only, and a combination group treated with BTVCs and MSZ. Treatment effectiveness, inflammatory response, and oxidative stress in the two groups before and after treatment were compared.

Results: The combination group had higher total effectiveness than the control group. The serum levels of MDA, high-sensitivity C-reactive protein (hs-CRP), TNF- α and interleukin-6 (IL-6) were lower, while serum levels of superoxide dismutase (SOD) and interleukin-10 (IL-10) were markedly increased in patients given combination treatment, when compared with controls. Pre-drug exposure UC disease activity index (UC-DAI) and clinical symptom scores were similar in both cohorts of patients, but the post-treatment scores were statistically decreased, especially in the combination group.

Conclusion: The combined use of MSZ and BTVCs was more effective against UC than monotherapy, as it effectively relieved inflammation and oxidative stress in patients, resulting in better clinical efficacy.

Keywords: MSZ, BTVCs, Ulcerative colitis, Inflammation, Oxidative stress

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INTRODUCTION

Ulcerative colitis (UC), a digestive disease with high incidence, is pervasive in young people aged 20 – 40 years [1]. The improvement of living standards of people in recent years has driven the ongoing incidence of UC in China. The occurrence of UC is attributed to multiple factors, but its mechanism remains elusive at present. In

light of its close relationship with inflammatory reactions, UC is considered an inflammatory disease [2]. Impaired scavenging of oxygen free radicals (OFR) in the body is also a key factor promoting ulcer formation, since increased levels of OFR enhance the activation of inflammatory mediators and induce an inflammatory response [3].

A positive microflora habitat in the intestinal microenvironment is conducive for the growth of large mass of microflora that maintains the mucosal barrier, aids digestion, and also influences various physiological processes in the body [4]. In recent years, a growing number of studies have linked this microenvironment to gastrointestinal diseases, since the gut microbiota is considered a vital organ of the body [5]. Increased levels of OFRs in patients lead to the activation of inflammatory mediators which in turn, enhance inflammatory reactions, damage tissues, and seriously affect the physical and mental health of patients. Currently, salicylic acid preparation [6] and sulfasalazine [7] are the main drugs used for the mitigation of inflammatory response in clinical practice. However, monotherapy of either drug usually fails to produce a satisfactory efficacy. Microecological preparation can bolster the intestinal environment, thereby increasing mucosal permeability which is of paramount importance in the treatment of UC.

Accordingly, this study applied combined use of MSZ and BTVCs for treating UC patients, and evaluated its curative effect and influence on inflammation and oxidative stress reaction in the patients.

METHODS

Patient profile

One hundred patients with UC who received in-patient or out-patient treatment in the Digestive System Department of our hospital from June 2019 to June 2020 were selected and allocated to combination group (n = 89; male-to-female ratio: 49:40, mean age: 43.5 ± 14.4 years) and control group (n = 11; male-to-female ratio: 9:2; mean age: 42.4 ± 19.2 years) using the random number table method. All the enrolled patients were diagnosed with UC which was confirmed via colonoscopy, pathology and clinical manifestations, with the lesion located in the rectum or sigmoid colon. The participation by all patients was without compulsion, and all participants submitted signed statements indicating their consent. Patients with drug allergy, blood coagulation disorder, peptic ulcer, surgical history, medication history involving hormones or immune-boosting preparations, as well as pregnant and lactating women, were excluded. Baseline data was similar in both groups ($p > 0.05$). The research protocol was approved by the Hospital Ethics Committee (approval no. 1846819L). The protocol used in this study followed the World Medical Association Declaration of Helsinki [8].

Treatments

The control group was given mesalazine (1 g at a time for a total of 4 times per day), while the combination group was, in addition, given oral administration of Bifid Triple Viable Capsules (420 mg half an hour after meals, twice for patients with a mild and moderate condition, and three times for patients with severe conditions. The course of treatment in both groups spanned 8 weeks.

Assessment of clinical efficacy

The clinical efficacy in patients was classified as *highly effective*, *effective* or *ineffective*. To be specific, *highly effective* indicated that after treatment, the clinical symptoms subsided; the intestinal mucosal ulcer tissue was healed, and stool test showed negative UC results. *Effective* indicated that after treatment, the clinical symptoms were significantly reduced; the intestinal mucosal ulcer tissue showed only light inflammation, while false polyp tissue could be seen, and the stool test result was negative. In contrast, *ineffective* indicated that after treatment, the clinical symptoms and intestinal mucosal ulcer tissue were not mitigated, and the stool test result was positive. Total response to treatment (TRT) was calculated as shown in Equation 1.

$$TRT (\%) = [(NS + Ne)/Nt] \times 100 \dots\dots\dots (1)$$

where Ns = number of *significantly effective* cases, Ne = number of *effective* cases, and Nt = total number of cases.

Determination of oxidative stress and inflammatory factors

Before and after treatment, 5 ml of elbow venous blood was collected from patients in both groups under fasting condition. The blood samples were centrifuged at 2000 rpm for 10 min to obtain sera. Serum hs-CRP was determined using radio immunoturbidimetry, while serum levels of IL-6, TNF- α and IL-10 were assayed using ELISA. Serum superoxide dismutase (SOD) was determined using the nitrite method, while serum malondialdehyde (MDA) was determined using the thiobarbituric acid (TBA) method.

Intestinal mucosal barrier function indicators

D-Lactic acid (D-LA) level was determined using D-lactic acid Colorimetric Assay Kit provided by eBioscience Inc. (San Diego, USA; item no. E-BC-K002-M). Diamine oxidase (DAO) was determined with Human Diamine Oxidase (DO)

ELISA Kit provided by eBioscience, Inc. (San Diego, USA; item no. PKSH032342. Endotoxin (ET) was determined with Human Endothelin 1 (ET-1) ELISA kit provided by eBioscience, Inc (San Diego, USA; Item no. E-EL-H0064c).

Evaluation of activity and clinical symptom score

The UC disease activity index (UC-DAI) was applied for the evaluation of disease activity before and after treatment, and clinical symptom scores were utilized for assessment of changes in clinical symptoms such as abdominal pain, diarrhea and mucus bloody stool before and after treatment.

Statistical analysis

Statistical analysis was done via SPSS 21.0. Measurement data are presented as mean \pm SD. Comparison was done with independent *t*-test, while count data were analyzed using χ^2 test. Significance of difference was assumed at $p < 0.05$.

RESULTS

Overall efficacy

As shown in Table 1, the combination group had a significantly higher overall efficacy (93.3 %) than the control group which had a value of 72.7 % ($p < 0.05$).

Table 1: Comparison of overall efficacy between the two groups

Group	Highly effective	Effective	Ineffective	Total effectiveness
Control (n=11)	5 (45.4)	3 (27.3)	3 (27.3)	8 (72.7)
Combination (n=89)	71 (79.8)	12 (13.5)	6 (6.7)	83 (93.3)
χ^2/t				5.0391
<i>P</i> -value				0.0248

Table 2: Levels of inflammatory factors in both groups (pg/mL, except for hs-CRP which is in mg/mL)

Variable	Control group (n=11)	Combination group (n=89)	<i>t</i>	<i>P</i> -value
hs-CRP				
Before	78.62 \pm 9.26	77.25 \pm 8.26	0.5123	0.610
After	41.25 \pm 5.26	25.61 \pm 2.64	16.2385	<0.001
TNF- α				
Before	35.62 \pm 4.25	34.25 \pm 3.91	1.0863	0.280
After	24.25 \pm 2.86	16.82 \pm 1.94	11.3246	<0.001
IL-6				
Before	197.62 \pm 22.51	193.51 \pm 20.61	0.6179	0.538
After	91.62 \pm 9.05	75.82 \pm 7.64	6.3416	<0.001
IL-10				
Before	25.12 \pm 2.36	26.01 \pm 2.45	2.1663	0.257
After	53.52 \pm 4.25	73.82 \pm 7.36	8.9383	<0.001

Oxidative stress indicators

In both groups, SOD activity and MDA content were similar before treatment, while SOD activity increased and MDA content decreased after treatment. Compared with the control group, the reduction in MDA content and the elevation in SOD activity were more evident in the combination group ($p < 0.05$; Figure 1).

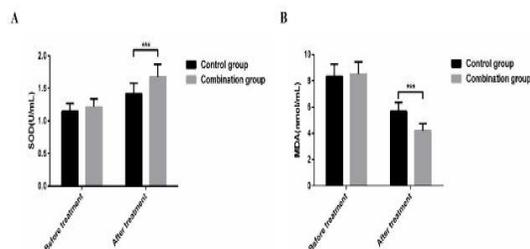


Figure 1: Comparison of oxidative stress indexes between two groups before and after treatment. (A) Comparison of SOD activity between the two groups before and after treatment; B: comparison of MDA content before and post-drug exposure. *** $P < 0.001$, when compared to control

Inflammatory factors

Before treatment, levels of inflammatory factors (hs-CRP, TNF- α , IL-6 and IL-10) were similar in the two groups. However, levels of hs-CRP, TNF- α and IL-6 were decreased, while IL-10 level was increased after treatment, with statistically significant differences between both groups (Table 2).

Indicators of intestinal mucosal barrier function

Table 3 shows that prior to treatment, no noteworthy difference was observed in intestinal mucosal barrier function between both groups, while post-treatment level of D-LA, DAO and ET were markedly reduced in combination group, relative to control.

UCDAI and clinical symptom scores

There were no marked differences in UCDAI and clinical symptom (diarrhea, abdominal pain and mucous bloody stool) scores before treatment between both series, but UCDAI and clinical symptom scores decreased significantly post-drug exposure, and the decrease was more severe in combination group ($p < 0.05$).

DISCUSSION

Ulcerative colitis (UC) has a long course of treatment and a high incidence of relapse. Delayed treatment of UC may result in deterioration which may lead to intestinal perforation [9] and cancer [10], which endanger the lives of people. To date, the pathogenesis of UC is considered to be related to persistent intestinal infection, abnormal immune regulation, and intestinal mucosal barrier dysfunction. These factors highlight the clinical significance of the control of mucosal microflora for reduction of

inflammatory reactions due to bacterial colonies [11].

Mesalazine, a 5-aminosalicylic acid preparation [12], is frequently used for UC treatment. It inhibits the secretion of colonic mucosa, decreases the secretion of prostaglandins and leukotrienes, inhibits the formation of OFRs, and suppresses the release of inflammatory mediators. It exerts a favorable anti-inflammatory effect, with few side effects, and it alleviates clinical symptoms such as abdominal pain, diarrhea, and pus and bloody stool, thereby restoring the colonic mucosa of patients [13,14]. Bifid Triple Viable Capsule (BTVVC) is a mixture of three kinds of probiotics, namely, *Bifidobacterium*, *Enterococcus* and *Lactobacillus acidophilus*. It can cross the gastric acid barrier and reach the intestinal tract directly, thereby supplementing normal beneficial bacteria groups in the intestinal tract. This contributes to improving the intestinal flora environment, maintaining the intestinal microecological balance, and reducing intestinal endotoxin production [15,16].

The results obtained in this study revealed no notable difference in levels of oxidative stress and inflammatory reaction factors between the two groups before treatment, but these indexes were markedly reduced in the combination group post-treatment, relative to control.

Table 3: Comparison of mucosal barrier indices between both groups

Variable	Control group (n=11)	Combination group (n=89)	t	P-value
D-lactic acid (mmol/L)				
Before	5.34±0.62	5.41±0.59	0.3693	0.713
After	4.68±0.41	4.11±0.38	4.6545	<0.001
DAO (U/L)				
Before	7.73±0.97	7.68±0.91	0.1707	0.865
After	6.54±0.87	5.41±0.59	5.6629	<0.001
ET (EU/mL)				
Before	0.24±0.09	0.25±0.07	0.4328	0.666
After	0.19±0.03	0.14±0.02	7.3665	<0.001

Table 4: Clinical symptom scores in the two groups

Variable	Control (n=11)	Combination (n=89)	t	P-value
UCDAI				
Before	7.62±1.02	7.57±0.94	0.1679	0.870
After	5.03±0.59	3.68±0.43	9.4087	<0.001
Diarrhea				
Before	2.71±0.61	2.66±0.63	0.2491	0.804
After	1.66±0.34	1.03±0.27	7.0919	<0.001
Abdominal pain				
Before	1.88±0.38	1.84±0.41	0.3075	0.759
After	1.20±0.26	0.88±0.11	7.5124	<0.001
Mucous bloody stool				
Before	4.42±0.68	4.40±0.55	0.1108	0.912
After	2.55±0.58	1.91±0.63	3.2036	0.002

The level of the inflammatory factor hs-CRP increases in different degrees in both active and remission stages of UC. Moreover, TNF- α is a critical pro-inflammatory cytokine which induces inflammatory reaction in the intestinal mucosa, leading to different degrees of injury in the intestinal mucosa. This underlies its important role in the pathogenesis of UC. In addition, IL-6 is a pro-inflammatory factor which induces pathological damage in colon mucosa and leads to inflammation of intestinal mucosa.

It has been reported that IL-10 is an inflammatory cytokine which activates monocytes and granulocytes to produce GM-CSF and G-CSF, and protects macrophages and neutrophils from chemotaxis [17]. In this study, after treatments, serum levels of hs-CRP, TNF- α and IL-6 were decreased significantly, while serum IL-10 showed a significant increase.

Lipid peroxidation is key causative factor for UC. An increase in OFR content causes an inflammatory reaction. In patients with UC, the MDA contents are increased, while the activity of the antioxidant enzyme SOD decreases, and the OFR scavenging is limited. These changes further aggravate the deterioration of the patient's condition.

The results of this study show that the combination group displayed lower MDA content and higher SOD activity than the control group, implying that the combination therapy mitigated oxidative stress in patients. During UC treatment, the use of combination of Bifid Triple Viable Capsules and mesalazine further enhanced the OFR scavenging capacity of the patients, reduced MDA concentration, increased SOD activity, and decreased levels of inflammatory factors. Moreover, the combination treatment inhibited intestinal inflammatory reactions, abated intestinal mucosal cell injury, improved intestinal function and enhanced clinical efficacy [18]. In this study, UCDAI and clinical symptom scores were decreased significantly after treatment, and the decreases were statistically greater in combination drug-treated patients than in controls, suggesting that combination of mesalazine and Bifid Triple Viable Capsules resulted in a promising performance in the treatment of UC.

Impairment of mucosal barrier of the intestine is a major presentation in UC. Therefore, the repair of this impairment in patients is the crux of UC treatment. Studies have shown that LA is a reliable index of intestinal permeability, while DAO is an effective index of intestinal mucosal structural integrity, and ET is a valid indicator of

intestinal function damage [19]. The present study revealed lower amounts of D-LA, DAO and ET in UC patients given combination treatment than in controls, suggesting that combination of BTVCs and mesalazine effectively regulated intestinal flora balance, reduced inflammatory factor infiltration and intestinal mucosal injury, and repaired intestinal mucosal barrier function.

CONCLUSION

The combined use of mesalazine and BTVCs significantly enhances treatment efficacy by mitigating inflammatory injury and oxidative stress reaction, as well as improving the intestinal micro-ecological environment of UC patients.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Lina Wei and Hua Xu conceived and designed the study, drafted the manuscript, collected, analyzed and interpreted the experimental data. Lina Wei revised the manuscript for important intellectual content. Both authors read and approved the final manuscript.

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