THE THERAPEUTIC EFFECTS OF BERBERINE EXTRACT ON ULCERATIVE COLITIS IN MICE

SI LI¹, YAN SHEN^{2*}

¹Department of Pediatrics, Tongde Hospital of Zhejiang Province, Hangzhou, 310012, China

²Department of Gastroenterology, the Second Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, 310005, China

*corresponding author: yanshenyanshen01@163.com

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Abstract

The aim of this study was to assess the effect and mechanism through which berberine extract (BBR) protects the colon mucosa mechanical barrier of mice suffering from ulcerative colitis (UC). 60 BALB/c mice were randomly divided into five groups, 12 mice per group: the normal control group (group A), the model control group (group B), the BBR low-dose group (group C - received 50 mg/kg BBR), the BBR high-dose group (group D - received 100 mg/kg BBR) and the salazosulfapyridine (SASP) positive control (group E - received 30 mg/kg SASP). The UC model was reproduced using dextran sulphate sodium (DSS). After the UC modelling, mice were treated for 7 days. The mice were observed daily for signs of morbidity and mortality along with the evaluation of disease activity index (DAI). After the last administration of the treatment, the mice were sacrificed and colon tissues were collected and stained with haematoxylin-eosin (HE) to observe pathological changes. Western Blot was used to quantify the levels of leucine-rich repeat-containing G-protein coupled receptor 5 (LGR-5) and telomerase reverse transcriptase (TERT) proteins and markers of intestinal stem cells (ISCs). The expression levels of claudin-1, occludin and zonula occludens-1 (ZO-1) were detected through immunohistochemical streptavidin peroxidase (SP) method and the expression levels of claudin-1 mRNA were measured through RT-PCR method. Compared to group B, the clinical and histopathological parameters of UC mice in each experimental group improved in different degrees, and the grade of DAI, macroscopic damage index (CMDI) and tissue damage index (TDI) in group D and E dropped significantly ($p < 0.01$). The content of both LGR-5 and TERT proteins in colon tissue of UC mice was significantly lower than that of normal mice ($p < 0.01$). After BBR or SSASP treatment, the LGR-5 and TERT protein content increased compared with model control group ($p < 0.05$). Expression levels of claudin-1, occludin and *zona occludens* proteins (ZO-1) in UC mice tissue were significantly lower than that of normal mice. Compared with group A, claudin-1 mRNA level increased in all four groups ($p < 0.01$). BBR has a beneficial effect on the onset and development of UC in mice. The mechanism of action probably involves the inhibition of ISCs and claudin degradation and maintaining the stability of colon mucosa mechanical barrier.

Rezumat

Scopul prezentului studiu a fost de a evalua efectul și mecanismul protector al extractului de berberină (BBR) asupra păstrării integrității barierei de protecție mecanică a mucoasei colonului la șoarecii cu colită ulcerativă (UC). 60 de șoareci BALB/c au fost împărțiți în 5 loturi de câte 12 animale în fiecare lot astfel: lotul de control normal (lot A), lotul de control al modelului (lot B), lotul BBR doză mică (lot C - 50 mg/kg BBR), lotul BBR doză mare (lot D - 100 mg/kg BBR) și lotul de control pozitiv al modelului tratat cu salazopirină (SASP) (lot E- SASP 30 mg/kg SASP). Modelul de UC a fost realizat prin administrare de dextran sulfat sodic (DSS). După realizarea modelului de UC șoarecii au primit tratament timp de 7 zile. Șoarecii au fost observați zilnic privind semnele de mortalitate și morbiditate și evaluați prin indexul de activitate a bolii (DAI). După ultima administrare a tratamentului, șoarecii au fost sacrificați, a fost recoltat colonul, iar modificările patologice au fost observate prin colorație hematoxilină-eozină (HE). Nivelul proteinelor LGR-5 (*leucine-rich repeat-containing G-protein coupled receptor 5*) și TERT (*telomerase reverse transcriptase*) și al markerilor pentru celulele stem intestinale (ISCs) au fost măsurate utilizând tehnica *western blot*. Nivelul expresiei claudin-1, occludin și a proteinei *zona occludens-1* (ZO-1) au fost determinate prin metoda imunohistochimică SP (*streptavidin peroxidase*), iar nivelul expresiei claudin-1 ARNm a fost determinat prin RT-PCR. Comparativ cu lotul B, parametrii clinici și histopatologici ai șoarecilor cu UC din loturile experimentale s-au îmbunătățit, iar valoarea DAI, a indicelui de afectare macroscopică (CMDI) și a indicelui de afectare a țesutului (TDI) în grupul D și E a scăzut considerabil ($p < 0,01$). Nivelul proteinelor LGR-5 și TERT de la nivelul colonului șoarecilor cu UC a fost semnificativ mai scăzut decât la șoarecii din lotul A ($p < 0,01$), iar acestea au crescut la valori comparative cu cele din grupul B după administrarea de BBR și SSASP ($p < 0,05$). Nivelurile proteinelor claudin-1, occludin și ZO-1 la șoarecii cu UC au fost semnificativ scăzute comparativ cu lotul A. Comparativ cu lotul A, nivelul claudin-1 ARNm în celelalte 4 loturi a fost semnificativ crescut ($p < 0,01$). BBR acționează ca un agent protector pentru apariția și progresia UC la șoareci. Mecanismul său de acțiune este, probabil, legat de inhibarea degradării claudin și a ISCs și menținerea stabilității mucoasei colonice.

Keywords: berberine extract, ulcerative colitis, mice, intestinal stem cells, claudin-1, occluding, ZO-1

Introduction

Ulcerative Colitis (UC) is a chronic and non-specific colitis disease, which has several clinic features such as perforation of colon, haemorrhage and infarction. UC has a high risk of malignancy and poor prognosis and has been recognized by World Health Organization (WHO) as a modern disease with poor treatment outcome [2, 22]. Studies carried out in recent years show that damage to the intestinal mucosa barrier and increasing permeability of the intestinal wall are the initial factors leading to UC morbidity and progression [1, 9, 14, 23]. In intestinal mucosa barrier system, mechanical barrier is one of the most important part, which consists of intestinal epithelial cells (IECs) and cell-cell junctions. IECs differentiate from intestinal stem cells (ISCs) and claudin is the key structural basis of cell-cell junctions. If mechanical barrier is damaged, antigens are translocated in the enteric cavity, thus leading to over-sustained immune inflammatory reaction and damages to the tissues.

Although synthetic drugs are the first treatment approach for UC, the herbal medicines and supplements are an alternative option. In recent years there is intense research on natural products because they are safer and have low incidence of side effects. The potency of natural products in treating and preventing from intestinal mucosa barrier system degradation have been extensively studied in animal studies [13, 27]. Berberine (BBR), also called *Rhizoma Coptidis*, is the active ingredient extracted from *Coptis chinensis* [12], a traditional Chinese medicine, which is cheap and widely used in medicine [3, 33, 36]. A variety of studies indicate that BBR is effective in treating experimental UC model, promotes regeneration and repair of damaged intestinal mucosa barrier, reduces permeability of intestinal wall and protects functions of intestinal barrier. But there are rare reports on the effect of BBR on intestinal mucosa barrier in UC. This study aimed to assess the therapeutic effect of BBR on colonic mucosal mechanical barrier in UC and explore the underlying mechanisms, in a murine model.

Materials and Methods

Reagents

The reagents used in the experiment were dextran sulphate sodium (DSS) (Sigma-Aldrich, Lot Number: 31404, Fluka: 5000MW); berberine hydrochloride (BBR) (from Sigma-Aldrich, Lot Number: 1065210, Mol. wt.: 371.81); salazosulfapyridine (SASP) (Sigma-Aldrich, Lot Number: 1636005, Mol. wt.: 398.39); Total Protein Extraction reagent (Thermo Pierce, Lot Number: 78510); 30% acrylamide solution (Bio-Rad, Lot Number: 161-0156); LGR5 and TERT primary antibody (Abcam, Lot Number: ab137484 and ab191523, Mol. wt.: 100 and 127); anti-rabbits and mice second antibodies (Thermo Pierce, Lot Number:

31160 and 31210); β -actin (Santa Cruz, Lot Number: SC-47778, Mol. wt.: 43).

Apparatus

Main instruments used were: paraffin slicing machine (Leica), fluorescence microscope camera with the type of BX20 (OLYMPUS), DTX880 multifunctional Microplate Reader (Beckman-Coulter), water-Jacket thermostat incubator (Jinghong Armamentarium Shanghai CO., Ltd.), UV spectrophotometer (Beckman-Coulter); high speed freezing centrifuge (Sigma), Mini-PROTEAN electrophoresis system and Trans-Blot rendition system (Bio-Rad) and Multiplex real-time quantitative fluorescence (Bio-Rad).

Preparation of drug suspension

High-dose and low-dose BBR suspension (50 mg/kg and 100 mg/kg) and SASP suspension were made by adding distilled water.

Animals

60 male healthy BALB/c mice of specific-pathogen-free (SPF) grade were purchased from Shanghai Lab Animal Research Center of Chinese Academy of Sciences. License number of animal production is SCXK (Shanghai) 2013-0006. The animals were 8 weeks old and mean weight of (20 ± 2) g. The animals were kept in specific animal cages in controlled room with temperature between 19 and 23°C, humidity between 35 and 55% and a 12-h light/dark cycle. The study was approved by the Ethical Committee of the Second Affiliated Hospital of Zhejiang Chinese Medical University.

The mice were divided into 5 groups including normal control group (group A), model control group (Group B), BBR low-dose group (group C: UC model and BBR of 50 mg/kg), BBR high-dose group (group D: UC model and BBR of 100 mg/kg) and positive control group (group E: UC model and SASP of 30 mg/kg), 12 mice *per* group. The animals were kept for acclimatization in standard environmental conditions: temperature $21 \pm 2^\circ\text{C}$, humidity between 35% and 55% and a 12-h light/dark cycle one week before the experiment started.

The UC model

The UC model was obtained by administration of DSS based on an improved method by Stevceva *et al* [24]. Briefly, 5% DSS solution was prepared (5 mg DSS was soluble in 100 mL PBS). Mice in group A had free access to drinking water and mice in other groups had free access to 5% DSS solution instead of drinking water for two weeks. After the two weeks exposure to DSS, one mouse *per* group was randomly chosen to confirm the model. Mice were sacrificed and celiotomized to collect the colons. Colon tissues were washed and observed for hyperaemia and oedema with naked eye. A length of 8 cm colon was used to perform histologic preparations to confirm the model.

From the next day after the model was developed, all mice were designed to drink normal water. Mice

stomachs were perfused with BBR or SASP suspension with 0.4 mL except group B and E. Mice in group E were treated with the corresponding drug solvent of equivalent volume once a day for seven days.

Animal evaluation

Prior sacrifice, behaviour of mice, feed and water consumption were observed and recorded. Each mouse was weighed regularly. The characteristics of stool and haematochezia were also observed and graded according to disease activity index (DAI, $DAI = \text{grade of body loss} + \text{grade of stool characteristics} + \text{grade of occult blood degree}$) [28].

The model was assessed in accordance with symptoms such as loose stools, blood-stained stools, weight loss and morphological changes.

Gross morphological examination of colon tissues

When medication was wrapped up, mice were sacrificed through cervical dislocation. Distal colon segments (8 cm from anus) were cut and harvested under strict aseptic conditions. Changes of mucous membrane and chorion were observed with naked eye. Colon macroscopic damage index (CMDI) was graded, as presented in Table I [21].

Table I

Grading of colon macroscopic damage index (CMDI)

Grade	Colon macroscopic damage
0	no damage
1	mild congestion and oedema; smooth surface without erosion and ulcer
2	congestion and oedema; rough and grainy mucous membrane with erosion
3	severe congestion and oedema; necrotic surface with ulcer: the highest vertical diameter is less than 1cm; intestinal wall thickened and the surface had necrosis and inflammation
4	severe congestion and oedema; necrotic surface with ulcer: the highest vertical diameter is not less 1cm; the intestinal wall was necrotic and megacolon, along with toxic megacolon, led to death

Ultrasonic examination of colon tissues

Fresh colon tissue was cut into pieces of 1.0 cm × 1.0 cm × 0.2 cm. It was put in an embedding box filled and fixed with 4% paraformaldehyde for 24 hours. It was then embedded with paraffin and cut

into slices with width of 3 - 4 μm. After haematoxylin-eosin (HE) staining, pathological changes of the tissue were observed under light microscope and tissue damage index (TDI) was graded as presented in Table II [6].

Table II

Grading of tissue damage index (TDI)

	Grade	Damage of mucous membrane
degree of pathology	0	no pathology
	1	pathology to mucous layer
	2	pathology to submucosa
	3	pathology to muscular coat
	4	pathology to serosa
pathology range	0	no pathology
	1	0% - 25%
	2	26% - 50%
	3	51% - 75%
	4	> 75%
inflammation degree	0	no inflammation
	1	mild
	2	moderate
	3	severe

Measurement of LGR-5 and TERT protein expression in colon tissues

The expression levels of intestinal stem cells (ISCs) biomarkers, namely LGR-5 (leucine-rich repeat containing G protein-coupled receptor) and TERT (telomerase reverse transcriptase) were measured through Western-blot analysis. Total proteins from colon tissues were extracted and the protein content was measured with bicinchoninic acid assay (BCA). 8 - 12% separation gel and 5% spacer gel were prepared. 60 μg protein was used for each group and 10 - 15 μL were pipetted in each hole. Samples were separated in 60 V separation gel and 80 V spacer

gel for approximately 2 hours. SDS-PAGE gel was balanced and proteins were wet transferred to the membrane under cooling condition with constant voltage of 100 V for 2 hours. The membrane was washed for one hour in T-TBS containing 5% bovine serum albumin (BSA). Next, tris-buffered saline (T-TBS) was used to wash the membrane three times and each time lasts 5 minutes. Primary antibodies of LGR-5, TERT and β-actin were used. Unbound primary antibody was washed with T-TBS five time for 5 minutes and the membrane was probed with the correspondent secondary antibodies. Unbound secondary antibody was washed with T-TBS five

time for 5 minutes. ECL was used to detect positive signal. After developing and fixing, software Band-Scan 5.0 was adopted to analyse optical density values of bands. The relative expression of the object protein: object protein (optical value)/internal reference (optical value) $\times 10$ n.

Examination of claudin-1, occludin and ZO-1 protein expression in colon tissues

Paraffin slices were attached to slide glasses treated with poly-lysine and then incubated at 60°C overnight. The distribution of claudin-1 in the tissue was determined by immunohistochemical SP (streptavidin-peroxidase) method. Positive stained were dyed brown. 5 visual fields were arbitrarily chosen to grade by using Fromowitz method. The specific grade standard used as described previously [31]: no colour is 0 point; light yellow 1 point; pale brown 2 points; brown 3 points; negative range: range less than 5% is 0 point; the range from 5% to 25% is considered as 1 point; 51% to 75% 3 points; range more than 75% 4 points. The two grades were added to get the result. The result less than 2 is

considered negative (-), 2 to 3 is the weakly positive (+), 4 to 5 is moderately positive (++) and 6 to 7 is strongly positive (+++).

Examination of claudin-1 mRNA expression in colon tissues

The levels of claudin-1 mRNA expression were detected by RT-PCR method. Total RNA in colon tissues was extracted with Trizol. Total RNA concentration was measured with a spectrophotometer by absorption at 260/280 nm and reverse transcription followed. The volume of the reaction of reverse transcription was 20 µL and the followed steps were according to kit specification. Reaction system of PCR amplification was 20 µL and primer sequences and reaction conditions are shown in Table III. PCR product was separated by agarose gel. Images were obtained in GEL imaging system.

Expression of target mRNA was compared to internal mouse glyceraldehyde-3-phosphated dehydrogenase (GAPDH). Relative expression of each gene was calculated as $2^{(C_{t_{\text{internal reference}}} - C_{t_{\text{object gene}}})}$.

Table III

Reaction system of PCR amplification, primer sequences and reaction conditions

Name of gene	Gene order number	Primer sequence	PCR reaction condition
Mouse Claudin1	NM_016674	5'-AGCACCGGGCAGATACAGT-3' 5'-GCCAATTACCATCAAGGCTCG-3'	95°C, 1 min, 40 circulation (95°C, 15 sec, 63°C, 25 sec, fluorescence collection)
Mouse GAPDH	GU214026.1	5'-GAAGGTCGGTGTGAACGGATTG-3' 5'-GAAGGTCGGTGTGAACGGATTG-3'	

GAPDH = glyceraldehyde-3-phosphated dehydrogenase

Statistical analysis

SPSS 17.0 software was used for statistical analysis and measurement data were expressed by mean $\pm$ standard deviation. Comparisons among groups were analysed by one-way ANOVA; comparisons between two groups were tested by LSD-method and checked by Dunnett test; and $p < 0.05$ indicated that the difference was statistically significant.

Results and Discussion

Normal conditions of mice and changes of disease activity

Mice in groups B-E had apparent symptoms including metal sluggishness, drumble, disordered fur, decrease of food intake, along with thin sloppy stool,

haematochezia and weight loss in different degrees. However, mice in group A had normal mental state, activity, fur, consumption of food and water, stool and weight levels. With the progress of medication, symptoms of mice in group C-E ameliorated and DAI in each also reduced compared with their condition before medication ($p < 0.01$). After medication, DAI in group D and E dropped significantly compared with that of group B in the same period ($p < 0.01$), while DAI in group C declined compared with group B, but the difference had no statistical significance ($p > 0.05$). Comparisons between group D and E showed no statistical significance ($p > 0.05$). Results are shown in Table IV.

Table IV

DAI grades before and after treatment (n = 12)

Group name	Before treatment	After treatment	
		3 rd day	7 th day
group A	0.07 $\pm$ 0.06 ^b	0.06 $\pm$ 0.05 ^b	0.07 $\pm$ 0.05 ^b
group B	1.89 $\pm$ 0.75 ^c	1.92 $\pm$ 0.81	1.74 $\pm$ 0.73
group C	1.90 $\pm$ 0.63 ^c	1.75 $\pm$ 0.86	1.42 $\pm$ 0.57 ^a
group D	1.94 $\pm$ 0.71 ^c	0.98 $\pm$ 0.55 ^{abc}	0.52 $\pm$ 0.40 ^{abc}
group E	1.85 $\pm$ 0.80	1.00 $\pm$ 0.49 ^{ab}	0.52 $\pm$ 0.46 ^{ab}

DAI grade differences were expressed comparatively with: ^a the values before the treatment ($p < 0.01$); ^b the values of group B in the same period ($p < 0.01$); ^c the values in group B in the same period, $p > 0.05$.

Gross morphological changes of mice colonic tissue

The macroscopic modifications of the mice colon included obvious ulcer nodules, blutene chloride/blood spots and vegetation. In addition, intestinal wall was soft, the content was thin and there were mucous membrane drops. Gross morphology of the mice colon in groups C-E improved obviously and the effect of BBR treatment was dose-dependent. Comparisons between groups D and C showed that ulcer symptoms ameliorated significantly, there were no ulcer nodules and the intestinal contents was melicera. CMDI of mice in group D and E decreased remarkably ($p < 0.01$) compared with mice in group B. Comparing groups D and E, it showed that CMDI levels did not differ significantly ($p > 0.05$). The results are shown in Table V.

Pathological changes of mice colon tissues

The microscopic examination of the colon tissues revealed that ulcerations widely occurred in colon mucosa of mice from group B. Tissues were congested and oedema was obvious. Part of intestinal wall was filled with disorganized cells and damaged mucous epithelium. Cell nucleus tended to confluence and they appeared blur. Cells fell off and intestinal villus was damaged. In tissues, there was an important infiltrate with inflammatory cells, most of which were neutrophil granulocytes. Ulcerations in group C reduced. Tissues were congested and oedema also alleviated. Cells

of mucous epithelium were organized and part of them fell off. There was still inflammatory cell infiltration; Ulcerations in group D and E relatively eased and tissue congestion and oedema also alleviated. Cells of mucous epithelium were relatively organized with cell rupture and inflammatory cell infiltration obviously lessening. It could be seen under the microscope that colon mucosa was complete and smooth, and the gland was clearly arranged and recognizable. There was no erosion, ulcer or neutrophil granulocyte infiltration. Compared with mice in group B, TDI in group D and E dropped remarkably ($p < 0.01$). The comparison of TDI between groups D and E shows that the difference had no statistical significance ($p > 0.05$). The results are shown in Table V and Figure 1.

Table V

CMDI and TDI grade in colon tissue of mice (n = 12)

Group name	CMDI	TDI
Group A	0.70 ± 0.49 ^a	4.11 ± 1.28 ^a
Group B	3.91 ± 1.37	9.03 ± 0.91
Group C	2.99 ± 1.12	7.74 ± 1.47
Group D	2.25 ± 1.09 ^{ab}	6.50 ± 1.06 ^{ab}
Group E	2.15 ± 0.98 ^a	6.27 ± 1.15 ^a

CMDI = colon macroscopic damage index, TDI = tissue damage index. The values were compared with: ^a group B ($p < 0.01$); ^b group E ($p > 0.05$).

Figure 1.

Pathological changes of colon tissue of mice ($\times 200$), where A: normal control group; B: model control group; C: BBR low-dose group; D: BBR high-dose group; E: positive control group

Expression level of LGR-5 and TERT protein in colon tissues of mice

Compared with group A, the expression level of LGR-5 and TERT protein in group B to E obviously decreased ($p < 0.01$). Compared with group B, the expression level of LGR-5 and TERT protein in group C to E increased significantly ($p < 0.05$); compared with group E the expression level of LGR-5 and TERT proteins in group C and D decreased significantly ($p < 0.01$). These results are presented in Table VI and Figure 2.

Table VI

Expression level of LGR-5 and TERT protein in colon tissues of mice (n = 12)

Group name	LGR-5	TERT
group A	12.85 ± 0.64 ^{cd}	11.50 ± 0.27 ^{cd}
group B	3.37 ± 0.22 ^{ad}	3.74 ± 0.28 ^{ad}
group C	4.69 ± 0.16 ^{acd}	4.33 ± 0.16 ^{abd}
group D	6.01 ± 0.15 ^{acd}	5.58 ± 0.20 ^{acd}
group E	9.12 ± 0.28 ^{ac}	7.90 ± 0.08 ^{ac}

Notes: compared with that of group A, ^a $p < 0.01$; compared with that of group B, ^b $p > 0.05$; compared with that of group E, ^d $p < 0.01$.

Figure 2.

Expression of LGR-5 and TERT protein colon tissue of mice

Expression level of claudin-1, occludin and ZO-1 protein in the colon tissue of mice

Compared with group A, the expression level of claudin-1, occludin and ZO-1 in groups B and C decreased significantly ($p < 0.01$), but no obvious changes were seen in groups D and E ($p > 0.05$).

Compared with group B, the expression levels in groups D and E were markedly increased ($p < 0.01$). The comparison between groups D and E showed no statistically significant difference ($p > 0.05$). The results can be found in Table VII and Figure 3.

Table VII

Expression level of claudin-1, occludin and ZO-1 protein in colon tissue of mice (n = 12)

Group name	Claudin-1	Occludin	ZO-1
Group A	5.79 ± 1.36^b	2.94 ± 0.52^b	4.33 ± 1.57^b
Group B	0.92 ± 0.70^a	0.68 ± 0.32^a	1.23 ± 0.68^a
Group C	1.14 ± 0.50^a	0.89 ± 0.30^a	1.15 ± 1.30^a
Group D	2.66 ± 1.05^{abc}	1.74 ± 1.26^{bc}	2.76 ± 1.54^{abc}
Group E	2.79 ± 1.41^{ab}	1.77 ± 0.80^b	2.43 ± 1.28^{ab}

Notes: compared with that of group A, ^a $p < 0.01$; compared with that of group B, ^b $p > 0.05$; compared with that of group E, ^c $p < 0.01$.

Figure 3.

Expression of claudin-1, occludin and ZO-1 protein in colon tissue of mice (SP, $\times 200$)

Relative expression level of claudin-1 mRNA in colon tissue of mice

Compared with group A, the expression level of claudin-1 mRNA in groups B to E decreased significantly ($p < 0.01$). Compared with group B, the expression level of claudin-1 mRNA in group C to E was significantly increased ($p < 0.01$). The comparison between groups D and E showed no statistical significant difference ($p > 0.05$). These results can be found in Table VIII.

Table VIII

Relative expression of claudin-1 mRNA in colon tissue of mice (n = 12)

Group name	Relative expression of claudin-1 mRNA
group A	1.010 ± 0.034^b
group B	0.122 ± 0.017^a
group C	0.341 ± 0.020^{ab}
group D	0.685 ± 0.049^{abc}
group E	0.726 ± 0.038^{ab}

The values were compared with: ^a group A ($p < 0.01$); ^b group B ($p > 0.05$); ^c group E ($p < 0.01$).

UC is one of the intractable diseases, which is common in western countries and its morbidity and incidence rate is increasing in recent years in China [18]. Currently, drugs commonly used for UC treatment are aminosalicic acid, glucocorticoids, immune-suppressants and biologicals, which can partially alleviate the symptoms. The high incidence of severe side effects of these drugs made long term treatment to be difficult to tolerate. Therefore, in order to know more about the pathology of UC, the core pathogenic mechanisms must be investigated. Exploring targeted

therapeutic measures, which can disrupt the disease progression and maintaining long-term treatment are the main focus of researcher to prevent severe and chronic UC.

The aetiology of UC is uncertain. It is believed that UC is the result of joint effect of factors such as immunity, heredity, environment, intestinal flora [29, 34]. Extensive research has shown that the gradual destruction of colon mucosa barrier and function obstacles are key players leading to clinical symptoms and pathological changes of colon. In the system of colon mucosa barrier system, mechanical barrier is the first and most important line consisting of intestinal endocrine cells (IECs) and cell-cell junctions. IECs include absorptive cell, beaker cell, Paneth's cell and neuroendocrine cell, which all differentiate from ISC in crypt base. Close connection is the major way for cell-cell junction, which is composed of three complete membrane proteins including occludin, claudins and junction adhesion molecule (JAMs) and peripheral cytoplasmic protein like ZO-1. It plays a key role in maintaining epithelial cell polarity and adjusting selectivity and permeability of the intestinal wall [17]. On the pathological state of UC, IECs apoptosis appears abnormally, toxic molecules and pathogenic microorganisms attack barrier structures so that barrier structures are destroyed leading to permeability of intestinal wall [20]; various antigens persistently stimulate the immune system of *laminae propria*. The latter releases several cell factors such as TNF- α and IL-1 β and enzymes like NF-KB to have immune inflammation attack, further leading to the damage of crypt, and reducing ISCs,

limitation to IECs updating, or non-function of IECs, which causes further damage to the intestinal epithelium. Mucosal lesion and immune inflammatory reaction are the major reasons for repeated occurrence of UC and complications of the disease are becoming more severe. Thus, the key to prevent severe and chronic UC is to maintain the colon mucosa mechanical barrier and improving the permeability of the intestinal wall [25]. Mucosal mending under the endoscope is widely considered as the critical prognostic factor and primary criterion for the end of treatment [7, 8]. In traditional Chinese medicine, UC is in the range of “dysentery (especially recurrent dysentery)”, “haematochezia”, “diarrhoea” and “haemorrhoidal haemorrhage” and its basic interpretation of the cause is damp abundance due to splenic asthenia [4]. Transporting and transforming function of the spleen and stomach lays the material foundation of non-born intestinal mucosa barrier, which is the important part of forming colon barrier function. Modern studies have also proved that on the condition of weak spleen, if colon mucosa barrier is destroyed and permeability rises, bacteria and toxic elements will invade in colon so that immune inflammatory reactions occur [30].

Berberine is the rhizome of *Coptis chinensis* with the features of bitterness and coldness, which can improve heart, liver, stomach and intestine condition. BBR is an isoquinoline alkaloid, with strong anti-inflammatory and immunomodulatory effects. Clinic and basic researches show that BBR can alleviate damages in intestinal mucosa caused by radiotherapy, non-steroid anti-inflammatory drugs and chronic stress [26, 32, 35], the available dose dependently reduces the permeability of retinal epithelial cells and protects its barrier function [10]. It can also decrease severe abdominal infection and the expression of inflammation mediators when pyaemia appear, thus improving the damaged intestinal mucosa barrier and reducing the permeability of intestinal wall [15, 19]. BBR is used in treating UC to promote the regenerating of intestinal mucosa and quicken the recovery of ulcer, which obviously reduces UC recurrence rate with accurate treatment effects [5, 11, 12, 16]. But there are rare reports of studies on its exact effect mechanism on intestinal mucosa barrier.

The experiment successfully developed mice UC model caused by DSS similar to human UC. Makers of ISCs including LGR-5 and TERT, and Claudin including claudin-1, occludin and ZO-1 were taken as the detection indexes to study protective function of BBR for colon mucosa mechanical barrier and its mechanism. Results showed that BBR can effectively ease some clinic symptoms such as weight loss, loose stool and haematochezia and obviously reduce DAI, CMDI and TDI grade ($p < 0.01$). It also has a good effect on pathological manifestations such as colonic

ulcer, damage to epithelium cells and infiltration of inflammatory cells. After the intervention treatment of high-dose BBR, DAI, CMDI and TDI grade of mice decrease remarkably ($p < 0.01$). The intervention effect is equivalent to SASP, positive control drug ($p < 0.05$), which displays great clinical effect. Also, the content of LGR-5 and TERT protein in colon tissue of UC mice decreased ($p < 0.01$). After the intervention treatment, the content increased ($p < 0.05$), but the intervention effect was weaker than that of positive control group ($p < 0.01$). Lastly, the expression level of claudin-1, occludin and ZO-1 in colon tissue of UC mice decreased ($p < 0.01$). However, after the intervention treatment, the expression level obviously increased ($p < 0.01$). Occludin levels of mice in high-dose BBR group tended to reach the value of normal mice. The effect of high-dose BBR on occludin was similar to that of the positive control SASP.

Conclusions

The present study suggests that the level of ISCs markers and occludin obviously decrease in UC model suggesting their implication in pathological mechanism of the damage of intestinal mucosa mechanical barrier and development of UC.

BBR can protect ISCs and occludin, the main components of intestinal mucosa mechanical barrier when UC occurs, and effectively cure colitis of mice, by inhibiting the damage of ISCs and occludin and maintaining the stability of intestinal mucosa mechanical barrier with good clinic effect.

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