Herb-partitioned moxibustion upregulated the expression of colonic epithelial tight junction-related proteins in Crohn’s disease model rats

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Abstract

Background: Herb-partitioned moxibustion (HPM) at Tianshu (ST25) and Qihai (RN6) has been used to treat Crohn's disease (CD). Injury to intestinal epithelial tight junctions (TJs) is the leading cause of CD onset with under expression of TJ-related proteins such as occludin, claudin-1, and zonula occludens protein-1 (ZO-1). This study aimed to investigate whether HPM can change the permeability of the intestinal epithelial barrier by affecting the expression of colonic epithelial TJ-related proteins in vitro.

Methods: Forty-eight male Sprague-Dawley rats were randomly divided into four groups of twelve rats: normal control (NC) group; model control (MC) group; herb-partitioned moxibustion (HPM) group; and mesalazine control (MESA) group. The rats in the latter three groups were given trinitrobenzene sulfonic acid (TNBS) enemas to establish CD models. The HPM group was treated with HPM at Tianshu (ST25) and Qihai (RN6) once daily for 14 consecutive days, while the MESA group was given mesalazine solution (at the proportion of 0.018:1) by lavage twice daily for the same period. After the treatment period, the colon tissues from all groups were partly processed for macroscopic damage assessment and histological observation, and partly purified and cultured in vitro to examine the permeability of the intestinal epithelial cell barrier by trans-epithelial electrical resistance (TEER). Western blot and fluorescence quantitative polymerase chain reaction (FQ-PCR) analyses were performed to observe the expression of occludin, claudin-1, and ZO-1 proteins and mRNAs, respectively.

Results: In the HPM and MESA groups, the typical CD macroscopic damage, i.e., inflammatory cell infiltration in colonic mucosa and submucosa, submucosal lymphoid follicular hyperplasia, hyperemia and edema, and morphological changes were improved to different degrees in the colonic tissues (HPM, MESA vs. MC for macroscopic score of colonic damage: all \(P < 0.001\)). The decreasing tendencies were minor for colonic TEER values (HPM, MESA vs. MC: all \(P < 0.001\)), and expression of intestinal epithelial TJ-related proteins (HPM, MESA vs. MC: all \(P < 0.05\)) and mRNAs (HPM, MESA vs. MC: all \(P < 0.05\)), especially in the HPM group (HPM vs. MESA for TEER values: \(P < 0.001\)).

Conclusions: HPM at Tianshu (ST25) and Qihai (RN6) upregulated the expression of occludin, claudin-1, and ZO-1 in TNBS-induced CD model rats.

Background

Crohn’s disease (CD) is a chronic inflammatory bowel disease (IBD) characterized by intestinal inflammatory changes induced by abnormal immune responses of the host against the intestinal tolerated commensal microflora [1, 2]. Injury to the intestinal epithelial barrier (e.g., excessive apoptosis of intestinal epithelial cells) and genetic defects in the intestinal epithelial tight junctions (TJs) (e.g., under expression of TJ-related proteins) were reported to be involved in the occurrence...
and development of CD [3]. Intestinal mucosal tumor necrosis factor-alpha (TNF-α) and tumor necrosis factor receptor1 (TNFR1) were overactive in patients with mild to moderate CD [4, 5]. Malformation and dysfunction of intestinal epithelial TJs were found in patients with IBDs [6, 7], and related to under expression or abnormal distribution of intestinal epithelial TJ-related proteins [8].

Intestinal epithelial TJs are related to the permeability of the intestinal epithelial barrier [9, 10]. Changes in the permeability of the intestinal epithelial barrier are the leading pathologic alterations in CD [11, 12]. Occludin, claudin-1, and zonula occludens protein-1 (ZO-1) are major proteins for maintaining TJ permeability [8]. During active and non-active periods of CD, the expression of occludin, claudin-1, ZO-1 proteins and mRNAs were significantly reduced in the colonic mucosa, followed by increasing permeability of the intestinal epithelium and injury to the intestinal epithelial barrier [13, 14].

The goals in CD treatment are remission of inflammation, recovery of mucosa, prevention of relapse, avoidance of surgical intervention and minimization of the possibility of cancer development [15]. 5-Aminosalicylates (5-ASA) and thiopurines are clinically used in treatments for IBD [16]. 5-ASA preparations (e.g., mesalazine) are the first-line medicines for IBD treatment in mild to moderate ulcerative colitis (UC) [17]. However, they were found to be no more effective than placebo for active CD [18]. Therefore, the European Crohn's and Colitis Organization has recognized the European Crohn’s and Colitis Organization has recognized the efficiency of mesalazine as “limited” [19]. Application of thiopurines in mild to moderate CD patients is limited because of their severe side effects including abnormalities in liver and renal functions, digestive intolerance and withdrawal reactions [20]. In consideration of this, alternatives that offer safe and highly effective treatment are urgently needed for patients with mild to moderate CD.

Previous clinical and experimental studies showed that herb-partitioned moxibustion (HPM) was effective in relieving abdominal pain, diarrhea and other gastrointestinal symptoms, as well as improving the Crohn’s disease activity index in patients [4, 5]. HPM was able to significantly reduce the abnormal increases in TNF-α and TNFR1 in the intestinal mucosa [5]. HPM not only decreased the apoptosis of colonic epithelial cells, but also enhanced the expression of colonic epithelial TJ-related proteins in vitro [21, 22].

This study aims to investigate whether HPM would change the permeability of the intestinal epithelial barrier by affecting the expression of occludin, claudin-1, and ZO-1 in vitro.

Methods

Animals

Forty-eight male Sprague-Dawley rats (150 ± 10 g), aged 6–8 weeks, were supplied by the Experimental Animal Center, Shanghai University of Traditional Chinese Medicine (China). The experiments were approved by Ethics Committee of Shanghai University of Traditional Chinese Medicine (No. 2013025; Additional files 1 and 2). Each animal was free of any known pathogens and only used once. All rats were housed in a light-controlled room (12-h/12-h light/dark cycle with lights on at 07:00 am), with constant temperature (22 ± 1°C) and humidity (42 ± 5%). Prior to modeling, the rats were housed with six companions in each cage, and had free access to food and water. The animal studies were performed following the ARRIVE guideline (Additional file 3).

The 48 male rats were allocated into four groups by a randomized block design. After weighing on a scale, the rats were ranked according to their body weight, and the numbers 1 through 48 were written on the tails of the rats in ascending order. According to this order, sets off our rats with similar approximate weights comprised one block, and were simultaneously given 48 random numbers taken from the random number table (from line 6 row 8, left to right). The four rats in each block were rearranged by their random numbers into ascending order, and then allocated into four groups of twelve rats: normal control (NC) group; model control (MC) group; herb-partitioned moxibustion (HPM) group; and mesalazine control (MESA) group.

The CD models were prepared according to Morris’ method [23]. Briefly, all rats were given only water for 24 h before modeling. After being weighed on the scale, the rats in all four groups were anesthetized with sodium pentobarbital (30 mg/kg 2 %; Sigma Chemical Co., USA) by intraperitoneal injection. The rats in the MC, HPM, and MESA groups were given trinitrobenzene sulfonic acid (TNBS; Sigma Chemical Co., USA) enemas (0.5 mL/100 g; formula: TNBS/50 % ethanol = 2:1), while those in the NC group were given enemas with the same dose of normal saline. The rats were fixed in the handstand position for 1 min while the rubber tubes containing the enema solution were retained in the gut cavity at 6–8 cm in depth to ensure an adequate drug action, and the tubes were then withdrawn. This procedure was repeated on days 7, 14, 21, and 28. When the CD modeling procedure was completed, two rats from each group were arbitrarily selected (by picking a number out of a box) to examine whether the modeling was sufficient by observing hematoxylin and eosinstained colonic tissues under a light microscope (Leica Microsystems GmbH, Germany).

Herbal cake and moxi cone preparation

The herbal powder formula was as follows: Fuzi (radix Aconiti carmichaeli) (10 g; Sichuan, China), Rougui (Cinnamomum cassia Presl) (2 g; Guanxi, China), Danshen
(radix *Salviae miltiorrhizae*) (3 g; Anhui, China), *Honghua* (*Carthamus tinctorius* L.) (3 g; Henan, China), and *Muxiang* (*Saussurea costus*) (2 g; Yunnan, China). Decoction pieces of these traditional Chinese medicines were mixed, ground into a fine powder, passed through a 150-mesh sieve three times, and stored in dry and dark conditions at room temperature (25 °C). For use, 2.5 g of herbal powder was fully mixed with 3 g of *Shaoxing* wine into a thick paste, and then shaped into herbal cakes (1 cm in diameter, 0.6 cm in height) in a herbal cake mold for the experimental rats.

Moxi cones (10 mg in weight, 0.6 cm in diameter, 0.6 cm in height) were made by hand using refined mugwort floss (Huatu, Suzhou, China).

**Treatments**

After excluding the rats arbitrarily selected from each group to examine whether the CD modeling was sufficient, the remaining 10 rats in each group were processed as described below.

All rats received the same fixation before treatment. The devices were adjusted until the rats felt comfortable and stopped struggling. When HPM was applied to the HPM group, the rats in the other three groups received the same fixation for the same duration.

The NC and MC groups received no treatment. For the HPM group, the fur of the lower abdomen was carefully shaved to expose the chosen acupoints, Tianshu (ST25) and Qihai (RN6). The moxa cones were ignited with an incense stick after placement on the herbal cake at Tianshu (ST25) and Qihai (RN6). Three moxa cones were used for each rat in a single treatment for 10 min. The rats received the same treatment once daily for 14 days. For the MESA group, mesalazine solution (Dr. Falk Pharma, Germany) at the proportion of 0.018:1 was fed by lavage [24]. The rats received the same treatment twice daily for 14 days.

**Assessment of macroscopic colonic damage**

After the treatment period, all rats in the different groups were euthanized with sodium pentobarbital (30 mg/kg 2%; Sigma Chemical Co., USA) by intraperitoneal injection. Colonic tissues (2–3 cm in length; 6–8 cm away from the anus) were taken and rinsed 5–10 times with sterile phosphate-buffered saline (PBS) containing antibiotics. Assessment of macroscopic colonic damage was conducted according to previously published criteria [25], as shown in Table 1. The severity of inflammation was assessed using the same criteria.

**Histological observation**

Colonic tissues (1 cm) were removed for histological observation. The tissues were washed in ice-cold normal saline, fixed in 10 % formalin, embedded in paraffin, cut into 4 μm thin sections, and adhered onto plain glass slides. The sections were then baked in an oven at 58 °C for 24 h for dewaxing, stained with hematoxylin and eosin, dehydrated in 95, 90, and 70 % ethanol, cleaned up in xylene, mounted in Permount or Histoclad, and observed at 200× magnification under an Olympus CX31 optical microscope (Olympus, Japan) at 2.80 V and 2.74 A. Images were obtained with a Canon A640 camera (Canon, Japan).

**Establishment of an in vitro intestinal epithelial barrier model**

The remaining tissues were cut into pieces with ophthalmic scissors, and digested with 0.4 % type IV collagenase for 2 h in an incubator at 37 °C and 5 % CO₂. The supernatants were centrifuged at 5000×g for 10 min. After the supernatants were discarded, the cell pellets were resuspended and seeded into culture dishes with Dulbecco’s modified Eagle’s medium (DMEM; Sigma-Aldrich Chemical Co., USA) containing 10 % fetal bovine serum (Sigma Chemical Co., USA) in an incubator at 37 °C and 5 % CO₂. The culture medium was changed every other day. Colonic epithelial cells were purified, digested with trypsin, and resuspended in complete medium before counting. Cells (1 × 10⁵) were taken and placed in 48-well plates for adherence and observation. Wells with high proportions of epithelial cells were expanded in culture. The above operations were repeated until most of the epithelial cells were visible under a light microscope (Leica Microsystems GmbH, Germany). The culture was continued for 7 days [26] until a complete epithelial barrier was formed from normal colonic epithelial cells.

**Table 1** Criterion for assessment of colonic damage

| Macroscopic score                           | Maximum score |
|---------------------------------------------|---------------|
| Adhesions                                    |               |
| None                                         | 0             |
| Minimal                                      | 1             |
| Involving several bowel loops                | 2             |
| Strictures                                   |               |
| None                                         | 0             |
| mild                                         | 2             |
| Severe, proximal dilatation                  | 3             |
| Ulcers                                       |               |
| None                                         | 0             |
| Linear ulceration < 1 cm                     | 1             |
| Two linear ulcers < 1 cm                     | 2             |
| More sites of ulceration or one large ulcer > 1 cm | 3             |
| Wall thickness                               |               |
| Less than 1 mm                               | 0             |
| 1–3 mm                                       | 1             |
| More than 1 mm                               | 2             |
| Maximum score                                | 10            |
TEER, western blot, and fluorescence quantitative PCR (FQ-PCR) analyses of colonic epithelial cells were performed in each group.

Measurement of colonic TEER
Cultured cells in the different groups were washed once with PBS and digested with 0.25 % trypsin [27, 28]. After adding culture medium and terminating the digestion process, the cells were counted and adjusted to a density of 2.5 × 10⁵ cells/mL. Transwell chambers for measuring transmembrane resistance were placed in 24-wellplates. Complete medium (600 µL) was added to the lower chamber, while a cell suspension (200 µL) was added to the upper chamber and cultured for 24 h at 37 °C and 5 % CO₂. The medium in the upper and lower chambers of the transwell chambers was replaced with 200 and 600 µL of medium, respectively. A small chamber with medium only was set as a negative control. The plates were measured in a Millicell ERS-2 Epithelial Volt-Ohm Meter (Millipore, USA) after vertical plug-in of an electrode into three slots on the chamber wall. The average of these three values was taken for statistical analysis. The room temperature was strictly controlled at 25 °C, because TEER values were reported to be highly sensitive to the environmental temperature [29].

Detection of occludin, claudin-1, and ZO-1 expression in the rat colonic epithelial cells cultured in vitro
Cells (2 × 10⁶) were taken from each group, added to a celllysis buffer (100 µL) until full cleavage was achieved, and boiled (100 °C) for 5 min. The insoluble material was removed by centrifugation (5000 for 5 min). The cellular proteins in each sample (50 µL) were separated by polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were sequentially blocked with 5 % nonfat milk in PBS for 2 h and 5 % bovine serum albumin in PBS at 4 °C overnight, and then incubated with primary antibodies for 90 min at room temperature. The primary antibodies used were rabbit polyclonal immunoglobulin (IgG) antibodies against occludin (Ab3172; Abcam, UK; 1:250 dilution), claudin-1 (Ab15098; Abcam, UK; 1:25 dilution), and ZO-1 (Sc-10804; Santa Cruz Biotechnology, USA; 1:100 dilution). Peroxidase-conjugated goat anti-rabbit IgG (A0208; Beyotime, China; 1:1000 dilution) and chemiluminescence fluids A and B (1:1) were incubated with the membranes for 1 h and 1 min, respectively. The reactive bands were detected by chemiluminescent reagents (170-5061; Bio-Rad, USA). A Tanon-5200 imager (Tanon, China) was employed for image acquisition, and Image-pro plus 6.0 software (MediaCybernetics, USA) was used to analyze the gray values of the bands. All the grey values of the bands were normalized by the value for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

FQ-PCR detection of occludin, claudin-1, and ZO-1 mRNA expression in the rat colonic epithelial cells cultured in vitro
Total cellular RNA was separated from the rat colonic epithelial cell samples using Trizol reagent (1596-026; Invitrogen, USA). Three micrograms of the total RNA was used as a template for reverse transcription of cDNAs. The cDNAs were synthesized by reverse transcription using a RevertAid cDNA Synthesis Kit (#K1622; Fermentas, USA) in accordance with the manufacturer’s instructions, and then amplified, the primer sequences used for PCR amplification reaction were shown in Table 2. The following cycling conditions were used: reverse transcription at 37 °C for 1 h, inactivation of the MMLV RT enzyme at 95 °C for 5 min, and 40 cycles of 50 °C for 2 min, 95 °C for 5 min, 95 °C for 15 s, and 60 °C for 45 s. Real-time PCR was performed with a Quantitect SYBR Green PCR Kit (#K0223; Thermo Fisher Scientific, USA) using an ABI 7500 real-time PCR system and SDS software (Applied Biosystems, USA). Data for the mRNA levels were calculated as the relative amounts normalized to that of GAPDH.

Statistical analysis
Statistical data were represented in graphs as mean ± standard deviation (SD). Statistical analyses were performed using SPSS 16.0 software (SPSS, USA). Differences within experimental groups were compared by one-way analysis of variance (ANOVA). LSD test was performed for multiple group comparison. Values of P < 0.05 were considered statistically significant.

Results
Colonic histopathological observations in the different groups
In the normal group (Fig. 1a), the colonic mucosa was intact, and the morphology of submucosal and muscular tissues was normal. In the MC group (Fig. 1b), there were large numbers of eosinophils with marked inflammatory cell infiltration in colonic mucosa and submucosa, and obvious submucosal lymphoid follicular hyperplasia, epithelial damage, submucosal hyperemia and edema, fibroblast proliferation, and muscle tissue damage, as well as epithelioid reactions, giant cells, and other typical pathological intestinal changes of CD. In the MESA group (Fig. 1c), there were eosinophils and inflammatory cell infiltration in colonic mucosa and submucosa, submucosal hyperemia and edema, fibroblast proliferation, small amounts of epithelioid reactions and giant cells,
and slightly disordered layers of tissue morphology. In the HPM group (Fig. 1d), there were only small amounts of inflammatory cell infiltration in colonic mucosa and submucosa, mild hyperemia, small amounts of fibroblast proliferation, and normal morphology of mucosal, submucosal, and muscular tissues.

Scores for macroscopic colonic damage in the different groups
Compared with the NC group, the MC, MESA, and HPM groups showed significant increases in the macroscopic scores for colonic damage ($P = 0.000$, $P = 0.000$, $P = 0.024$; Fig. 2). However, the macroscopic scores for colonic damage in the MESA and HPM groups were significantly lower than that in the MC group ($P = 0.000$, $P = 0.000$), although there was no significant difference between these two groups ($P = 0.075$). Both HPM and MESA therapy could significantly improve the macroscopic colonic mucosal damage in CD.

Comparison of TEER values in the different groups
As shown in Fig. 3, there was a significant decrease in the colonic TEER values in the MC group compared with the NC group ($P = 0.000$). There were significant increases in the colonic TEER values in both the HPM and MESA groups compared with the MC group ($P = 0.000$, $P = 0.000$).

Table 2: The sequence of the forward primer and reverse primer used for FQ-PCR assays

| Name   | Primer sense | Primer sequence (5′ → 3′)                     | Amplification product (bp) |
|--------|--------------|-----------------------------------------------|-----------------------------|
| GAPDH  | Forward      | $5′$-CCGAGGGCCCACTAAAGG-3′                     | 116                         |
|        | Reverse      | $5′$-GCTGTTGAAGTCAAGAACCA-3′                  |                             |
| Occludin| Forward      | $5′$-GGGACAGAGCCTATGGA-3′                     | 191                         |
|        | Reverse      | $5′$-GGAAGGATGAAGCAAA-3′                      |                             |
| Claudin-1| Forward     | $5′$-CTGGCCTCGCCTGGGATGGA-3′                  | 100                         |
|        | Reverse      | $5′$-TGGGCTGAGCAGTCACGATGTT-3′                |                             |
| ZO-1   | Forward      | $5′$-GATGAGCGGCTACCTTA-3′                     | 126                         |
|        | Reverse      | $5′$-ATGGGAGCGAACTGATG-3′                     |                             |

Morphological observation of colonic tissues

Fig. 1 Histopathological observation of the colon tissue. Hematoxylin and eosin staining method, ×200. a NC group; b MC group; c MESA group; d HPM group

Fig. 2 Macroscopic score of colonic damage. NC normal control group, MC model control group, MESA mesalazine group, and HPM herb-partitioned moxibustion group. ★$P < 0.001$ versus NC, ★★★$P < 0.005$ versus NC, △$P < 0.001$ versus NC. The data of macroscopical score do not have normal distribution, the box-plot is used to express, as shown below. The NC group has the same value, so the box-plot only have one straight line. The maximum value is equal to the upper quantile and the minimum value is equal to the lower quantile in MC group. The maximum value is equal to the upper quantile in MESA group. The maximum value is equal to the upper quantile and the minimum value is equal to the lower quantile in HPM group.
Expression of occludin, claudin-1, and ZO-1 proteins in the different groups

For occludin protein expression (Fig. 4a), there was a significant decrease in the MC group compared with the NC group \((P = 0.000)\), significant increases in the HPM and MESA groups compared with the MC group \((P = 0.025, P = 0.019)\), and no significant difference between the HPM group and the MESA group \((P = 0.859)\), but significant differences in the HPM and MESA groups compared with the NC group \((P = 0.001, P = 0.000)\). For claudin-1 protein expression (Fig. 4b), there was a significant decrease in the MC group compared with the NC group \((P = 0.000)\), significant increases in the HPM and MESA groups compared with the MC group \((P = 0.001, P = 0.002)\), no significant difference between the HPM group and the MESA group \((P = 0.535)\), and no significant differences in the HPM and MESA groups compared with the NC group \((P = 0.196, P = 0.073)\). For ZO-1 protein expression (Fig. 4c), there was a significant decrease in the MC group compared with the NC group \((P = 0.008)\), significant increases in the HPM and MESA groups compared with the MC group \((P = 0.038, P = 0.046)\), no significant difference between the HPM group and the MESA group \((P = 0.899)\), and no significant differences in the HPM and MESA groups compared with the NC group \((P = 0.336, P = 0.282)\).

Discussion

This study has demonstrated the effects of HPM on repairing the colonic epithelial barrier and upregulating the expression of occludin, claudin-1, and ZO-1 in vitro. In the study, we first observed both macroscopic and histological damage to the colonic tissues from the different groups. Immediately after the colonic tissues were removed and washed, we assessed the scores for adhesions, strictures, ulcers, and wall thickness of the colonic tissues. In the NC group, the colonic tissues showed mild hyperemia and edema, but no mucosal erosion or ulcers, which might have arisen through stimulation during the surgery process. Almost no signs of tissue adhesions and strictures were observed, and the wall thickness was normal. In the MC group, severe hyperemia and edema were generally observed in most rats, while deep and multiple ulcers were common. In several rats, we also observed slight adhesions of the adjacent colonic tissues, accompanied by loss of elasticity and smoothness of colonic tissues, and slight increases in wall thickness. This was because that our CD modeling procedure could only imitate the incipient acute colonic inflammation, which is characterized by mucosal hyperemia, edema, and ulcers. However, the symptoms of tissue adhesions, strictures,
and intestinal wall stiffness were mainly the results of chronic recurrent inflammation. Compared with the MC group, the MESA and HPM groups showed remarkable signs of inflammation remission, as the mucosal hypere-aemia, edema, and ulcers were moderate and localized, and adhesions and strictures were barely observed.

By light microscopy, we observed pathological changes of the colonic tissues in the CD, HPM, and MESA groups. In tissues taken from the MC group, eosinophils and inflammatory cell infiltration in colonic mucosa and submucosa, and obvious submucosal hyperemia and edema, as well as obvious lymphoid follicles, fibroblast proliferation, and other typical CD pathological changes were observed under a light microscope. After treatment with mesalazine, these pathological changes were not improved. After treatment with HPM, there was only a small amount of inflammatory cell infiltration in colonic mucosa and submucosa, mild hyperemia, and scattered fibroblast proliferation among normal morphology of the mucosal, submucosal, and muscular tissues.

We then examined the TEER of the in vitro cultured colonic epithelial cells from the different groups. TEER determination is widely used to functionally analyze TJ dynamics in cell culture models of physiological barriers.
which can be used to evaluate the paracellular permeability of epithelial monolayers. A decrease in TEER was associated with disruption of TJs and subtle changes in the cytoskeletal structure, resulting in a subsequent increase in the paracellular permeability of the epithelial monolayer [33]. In the present study, we found significant decreases in the TEER values in the MC group, but increases to different degrees in the HPM and MESA groups, with the HPM group showing larger rising trends. These findings suggested that during the pathological process of CD, the structure of TJs was impaired, leading to a subsequent decrease in the paracellular permeability of the epithelial monolayer. However, both HPM and mesalazine treatment can recover or reduce the abnormally increased colonic epithelial permeability in CD, and HPM possesses the advantages of repairing TJs and increasing the permeability of the epithelial monolayer.

The intestinal epithelium creates a chemical and mechanical barrier that separates the host from microbes [34]. The chemical barrier consists of mucus, peptides, and antimicrobial proteins secreted by goblet cells and Paneth cells, while the mechanical barrier is composed of different kinds of enterocytes and TJs to join the cells together. These barriers are responsible for permeability, i.e., selective absorption of water and nutrients in the gut lumen through either transepithelial or paracellular transport. TJs are multimolecular complexes that seal the intercellular space between adjacent epithelial cells [35] and act as a diffusion barrier to control the transportation of ions, macromolecules, and immune cells in the paracellular pathway [36], and are therefore the major determinants of epithelial permeability [37]. TJs maintain symbiosis within intestinal microflora and prevent infection or inflammation induced by bacterial translocation [38].

The TJ-related proteins of the intestinal epithelial barrier are mainly located at the top side of the enteric cavity among intestinal epithelial cells, then zonally surround the side wall and seal up the intestinal epithelial gap. TJs are composed of three transmembrane protein families [39]: claudins, junctional adhesion molecule (JAM) proteins, and TJ-associated Marvel domain proteins (TAMPs) including occludin, tricellulin, and Marvel D3. Claudins control the charge and size-selectivity of TJs, while JAMs and TAMPs stabilize TJs and maintain epithelial permeability. ZO-1 connects the multiprotein structure of TJs to peri-junctional actomyosin. These three protein families have overlapping and unique functions in the regulation of TJs [40]. In this study, occludin, claudin-1, and ZO-1 were formed into complex proteins by binding between the C-terminals of the TJ transmembrane proteins claudin-1 and occludin and

![Fig. 5](image-url)  
**Fig. 5** mRNA levels of colonic epithelial TJ-related protein occludin, claudin-1 and ZO-1 in each group. **a** mRNA levels of occludin in each group. **b** mRNA levels of claudin-1 in each group. **c** mRNA levels of ZO-1 in each group. **NC** normal control group, **MC** model control group, **MESA** mesalazine group, and **HPM** herb-partitioned moxibus-tion group. *P* < 0.01 versus NC; **P** < 0.05 versus NC; ##P < 0.05 versus MC.
the N-terminals of the cytoplasmic-attached protein ZO-1 [41], thus allowing their external parts to interact with the transmembrane junction proteins of adjacent cells. At the same time, the C-terminals of ZO-1 were connected with actin of the cytoplasmic cytoskeleton, making it possible for claudin-1 to act on these actin molecules, and thus participate in maintaining the stability and selective permeability of TJs [42]. Occludin is considered to be fundamental to the structure of TJs [43]. Knockout of the occludin gene [28] in epithelial cells can cause similar pathological intestinal changes to IBD in animals. Reduced expression of intestinal occludin protein and mRNA was observed in patients with ulcerative colitis and CD [3], suggesting the possible involvement of occludin in the pathogenesis of IBD. In the present study, in vitro experiments demonstrated radical decreases in colonic epithelial occludin protein and mRNA expression in the NC group, compared with remarkable recovery of the occludin protein and mRNA expression in the MESA and HPM groups. The same tendency was observed for the other two TJ-related proteins, claudin-1 and ZO-1.

Conclusions

HPM at Tianshu (ST25) and Qihai (RN6) upregulated the expression of occludin, claudin-1, and ZO-1 in TNBS-induced CD model rats.

Additional files

- Additional file 1. Ethical approval document in Chinese.
- Additional file 2. Ethical approval documents in English.
- Additional file 3. The ARRIVE guidelines.

Abbreviations

CD: Crohn’s disease; IBD: inflammatory bowel disease; TJ: tight junction; TNF-α: tumor necrosis factor-alpha; TNFR1: tumor necrosis factor receptor-1; ZO-1: zonula occludens proteins-1; UC: ulcerative colitis; ECCO: European Crohn’s and Colitis Organization; SD: Sprague–Dawley; TNBS: trinitrobenzene sulfonic acid; NC: normal control; MC: model control; HPM: herb-partitioned moxibustion; RT: room temperature; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; MESA: mesalazine; FQ-PCR: fluorescent quantitative polymerase chain reaction; SD: standard deviation; ANOVA: one way analysis of variance; JAM: junctional adhesion molecule; TAMPs: TJ-associated Marvel domain proteins.

Authors’ contributions

YS conceived and designed the study. RJ performed measurement of colonic TEER and cell culture. AQW performed the assessment of macroscopic colonic damage and Western blot. HXS performed FQ-PCR detection and cell culture. LC performed histological observation. HXS, LC, CHB and LYW performed data acquisition, analysis and interpretation. RJ and AQW wrote the manuscript. HGW revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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