Effect of Tongxieyaofang decoction on colonic mucosal protein expression profiles in rats with visceral hypersensitivity

Lin Yongjun, Ding Ying, Lü Bin, Liu Nan

Lin Yongjun, Liu Nan, Department of Intensive Care Unit, Sir Run Run Shaw Hospital, Medicine School of Zhejiang University, Hangzhou 310016, China
Ding Ying, Department of Intensive Care Unit, Sir Run Run Shaw Hospital XiaSha Campus, Medicine School of Zhejiang University, Hangzhou 310018, China
Lü Bin, Department of Gastroenterology, the First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310006, China
Correspondence to: Lin Yongjun, Department of Intensive Care Unit, Sir Run Run Shaw Hospital, Medicine School of Zhejiang University, Hangzhou 310018, China.
Telephone: +86-571-86006846
Accepted: February 23, 2019

Abstract

OBJECTIVE: To explore the underlying mechanism of action of Tongxieyaofang decoction in rats with visceral hypersensitivity using proteomics technology.

METHODS: Twenty-four female Sprague-Dawley rats were randomly divided into three groups: control group, irritable bowel syndrome (IBS) group and Tongxieyaofang treatment group. An IBS model, characterized as visceral hypersensitivity, was established using the odour of mothballs as conditional stimulation and colorectal distension combined with classic physical restraint as non-conditional stimulation. Rats were intragastrically treated with Tongxieyaofang (2 or 4 mL · kg⁻¹ · d⁻¹) for 4 weeks. On the 45th day, the rats were dissected and the colonic mucosal proteins were extracted. Differential protein spots were screened by fluorescent two-dimensional differential gel electrophoresis (2D-DIGE), and identified by matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF-MS). Western blotting experiments were performed to verify the changes observed in 2D-DIGE and MALDI-TOF-MS.

RESULTS: It was found that the visceral sensitivity of rats in the Tongxieyaofang treatment group (4 mL/kg) was lower than that in the IBS group (P < 0.01). Sixty-one protein spots were differentially expressed between the IBS group and the Tongxieyaofang treatment group. Of these, 23 spots were upregulated in the Tongxieyaofang treatment group, while 38 spots were downregulated. Three specific proteins were successfully identified from the five protein spots with the most obvious changes. The two upregulated proteins were transgelin (TAGLN) and acetaldehyde dehydrogenase 2 (Aldh2) and the downregulated protein was cytokeratin 8 (CK8).

CONCLUSION: Tongxieyaofang can dose-dependently ameliorate visceral hypersensitivity in rats and the mechanism of action may involve the upregulation of TAGLN and Aldh2 and the downregulated protein was cytokeratin 8 (CK8).

© 2020 JTCM. All rights reserved.

Keywords: Irritable bowel syndrome; Hypersensitivity; Acetaldehyde dehydrogenase inhibitor; Keratin-8; Tongxieyaofang

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder, with a prevalence in Western
countries as high as 10% to 20%. Conventionally, IBS is considered a cluster of multiple symptoms, such as pain, diarrhoea or constipation, which significantly impact patients’ health-related quality of life. Although the mechanism of IBS may involve various factors, such as dysregulation of the brain-gut axis, abnormal intestinal motility, altered bile metabolism, immune dysfunction, paraesthesia, infection, low-grade inflammation of the intestine, small intestinal bacterial overgrowth and cytokine gene polymorphisms, the pathogenesis of IBS has still not been fully elucidated. In addition to lifestyle changes and dietary modifications, pharmacotherapy is an important part of IBS treatment. However, the effect of most drugs is modest and only focuses on certain troublesome symptoms, and the side effects that accompany these drugs lower their safety. Therefore, more effective pharmacotherapy is urgently needed. Tongxieyaofang, a Chinese herbal formula that comprises rhizoma atractylodis macrocephalae, tangerine peel, white peony root and radix sileris, has been widely used for painful diarrhoea associated with IBS. To date, the mechanism by which Tongxieyaofang alleviates painful diarrhoea remains unclear. It has been reported that Tongxieyaofang may inhibit the expression of protease activated receptor 2 (PAR-2), a serine protease receptor, reducing the levels of substance P, interleukin (IL)-6 and tumour necrosis factor (TNF)-α in the colonic mucosa and decreasing faecal serine protease activity. However, further research is needed to fully elucidate the mechanism underlying the activity of Tongxieyaofang. Here, to explore the possible mechanisms of Tongxieyaofang in the treatment of IBS, we used proteomics technology to observe the effects of Tongxieyaofang on the protein expression profiles in the visceral colon mucosa of rats with IBS. Tongxieyaofang was found to promote the expression of transglamin (TAGLN) and acetdehyde dehydrogenase 2 (Aldh2), and inhibit the expression of cytokeratin 8 (CK8). Our findings may provide novel insight into the mechanisms underlying the therapeutic effects of Tongxieyaofang against IBS.

MATERIALS AND METHODS

Animals

Twenty-four adult female Sprague-Dawley (SD) rats (220–250 g, two-month-old) of specific pathogen free grade were obtained from the Animal Centre of Zhejiang Chinese Medical University (Hangzhou, China, SYXX (Zhe) 2013-0184). Pairs of animals were housed in steel hanging cages with temperature and humidity maintained at (22 ± 1) °C and 50% ± 20%, respectively, with alternate 12-h periods of light and dark. The rats were provided with commercial feed and tap water ad libitum. The animal experimental protocols were approved in accordance with the guide for the Care and Use of Laboratory Animals prepared by the Institution-Animal Care and Use Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University.

Preparation of the Tongxieyaofang decoction

Based on an adult body weight of 60 kg, the formula for Tongxieyaofang (as prescribed in the First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine) includes 15 g of roasted Baizhu (Rhizoma Atractylodis Macrocephalae) 12 g of Baishao (Radix Paeoniae Alba), 10 g of Fangfeng (Radix Saposhnikoviae) and 6 g of Chenpi (Pericarpium Citri Reticulatae). The rat dosage used was 6.25 times that of the normal human dosage (per kg body weight). The crude drug granules were dissolved in distilled water to give a concentration of 1.0 g/mL. The drug was stored in a drinking bottle at 40 °C in the dark prior to administration of 4.5 · kg⁻¹ · d⁻¹ of crude drug, i.e. the decoction.

Reagents

Coomassie brilliant blue R-250, dithiothreitol (DTT), urea, agarose, glycerine, bromophenol blue, 3-[(cholamidopropyl)dimethylammonio]-1-propanesulphophosphate (CHAPC), Tris, glycine, iodine acetamide (IAA), immobilised pH gradient (IPG) strips (pH 3-10) and a Bradford protein quantitative kit were purchased from Bio-Rad Co., Ltd. (Hercules, Chicago, IL, USA). The manufacturers of other reagents used in this study were as follows: thiourea (Fluka Co., Ltd., St. Louis, MO, USA); protease inhibitor cocktail (Roche Co., Ltd., Basel, Switzerland); CyDye fluorescent dye (GE Healthcare Co., Ltd., Pittsburgh, PA, USA); lysine (Amresco Co. Ltd., Solon, OH, USA); and Milli-Q water (Millipore Co., Ltd., Burlington, MA, USA). The lysis buffer (400 mL) consisted of 7 mol/L of urea, 2 mol/L of thiourea, 4% CHAPS and 30 mM Tris (pH 8.5). The rehydration buffer consisted of 7 M urea, 2 M thiourea, 4% CHAPS, 1% IPG buffer, 40 mmol/L DTT and bromophenol blue. The equilibration buffer consisted of 6 mol/L urea, 2% sodium dodecyl sulphate, 50 mM Tris-Cl (pH 8.8) and 30% glycerol. The constant volume of Milli-Q water was 500 mL. Antibodies against TAGLN, Aldh2 and CK8 were purchased from Santa Cruz Biotechnology (Santa Cruz, Chicago, IL, USA).

Instruments

The primary instruments used in this study included the following: the Etan IPGphor I Isoelectric Focusing Instrument and Etan DALT® Six Large-scale Vertical Electrophoresis System (Applied GE Healthcare, Pittsburgh, PA, USA); a Typhoon 9410 scanner; an Etan Spot Picker Automatic Spot Chipped System and Etan Spotter Automatic Mass Spectrum Sample System (Applied Amersham Biosciences, Piscataway, NJ, USA); 4800 Plus Matrix-assisted laser desorption/ionisation time-of-flight (MALDI TOF/TOF) Instrument (Applied Biosystems Co., Ltd., Foster City, CA, USA); and a 400 R table-top centrifuge (Applied Heraeus Co., Ltd., Hanau, Germany).
Induction of IBS and treatment

Twenty-four SD rats in our experiment were randomly separated into three groups (eight per group): the control group, the IBS group and the Tongxieyaofang treatment group. The control group was fed under normal conditions. The IBS group and treatment groups were treated as described previously.9 Briefly, the rats underwent isobaric colorectal distension (CRD; 60 mm Hg for 10 min, twice, with a 10-min rest interval) using a pressure control device (barostat; Astra Zeneca, Möln达尔, Sweden), between 9:00 and 9:30 am daily for 8 d. Tonic CRD was applied for 3 to 10 min within noxious and non-noxious ranges. After successful preparation of the models, each group was given a different drug intervention. The rats in the control and model groups were administered intragastrically with 0.9% NaCl solution (4 mL·kg⁻¹·d⁻¹) for 4 weeks, while the treatment group received Tongxieyaofang decoction (2 or 4 mL·kg⁻¹·d⁻¹) intragastrically for 4 weeks.

Behavioural testing

Behavioural testing following CRD was carried out in accordance with a previous study.9 Briefly, behavioural responses were assessed in the IBS group and the Tongxieyaofang treatment group for 7 d after cessation of the irritation protocol by measuring the abdominal withdrawal reflex (AWR). Rats were evaluated by the semi-quantitative AWR test as follows: 0: no behavioural response to CRD; 1: brief head movement only; 2: contraction of abdominal muscles; 3: lifting of abdomen; and 4: body arching and lifting of pelvis. The rats from the IBS group and Tongxieyaofang treatment group were lightly sedated by ether and an infant catheter balloon was placed intra-rectally and secured on the tail. The proximal and distal ends of the balloon resided in the descending colon and the anus, respectively. The rats were woken and adapted for about 20 min in small Lucite cubicles (20 cm × 8 cm × 8 cm) on an elevated Plexiglas platform. The balloon was then expanded gradually by water injection. The balloon volume at which each rat responded by lifting the abdomen (AWR score of 3) was recorded. The entire process was repeated three times for statistical analysis.

Visceral sensitivity assessment

On the 43 rd day of the experiment, a rectal injection expansion experiment was performed on each animal, using the AWR score evaluation, rat visceral sensitivity and specific operation methods as previously stated.

Enzyme-linked immunosorbent assay (ELISA) analysis

The amounts of 5-hydroxytryptamine (5-HT), substance P (SP) and vasoactive intestinal peptide (VIP) in plasma were quantified by ELISA kits according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA).

Histology analysis

For histological analysis, formalin-fixed paraffin-embedded segments of colon tissue were sectioned at 5 µm in thickness and the sections were stained with haematoxylin and eosin (HE).

Protein preparation

After the 45th day of the experiment, the rats from the three groups were dissected and the proximal 2 cm of the descending colon was excised. The segments were washed with phosphate-buffered saline (PBS) containing protease inhibitor cocktail and opened longitudinally as previously reported.9 For each sample, the mucosa was separated from the muscle layer using a sterile sharp blade and was stored at −80 °C to avoid contamination. The frozen tissues were washed twice with cold PBS. Colon tissue from the eight rats in each group was pooled and crushed using a pestle in liquid nitrogen. Lysis buffer containing 2 mM dithiothreitol (DTT) was added to 100 mg of tissue. The mixture was sonicated on ice and then centrifuged at 4 °C for 5 min at 15 000 × g. Then, the supernatant containing the tissue protein was collected and purified using a 2-D Clean-Up kit (GE Healthcare Amersham Biosciences, Uppsala, Sweden). The pH of the protein samples was adjusted to 8.5 with 50 mM NaOH. The protein sample concentration was determined according to the Bradford method (BioRad Protein Assay, BioRad) in accordance with our previous report.17

Fluorescent labelling of protein samples

The pH values of the samples from each group were adjusted to 8.5 by the addition of 50 mmol/L NaOH. In the dark, the control group samples (50 µg, 5-10 µL) were labelled with 1 µL (400 pmol) of CyDye Cy3. The IBS group samples (50 µg, 5-10 µL) were labelled with 1 µL (400 pmol) of CyDye Cy5, and the pooled samples from the control group and the IBS group (25 µg of each) were labelled with 1 µL (400 pmol) of CyDye Cy2 (as internal standard 1). An additional reverse-labelling experiment was conducted, i.e. the control group samples were labelled with CyDye Cy5, and the IBS group samples were labelled with CyDye Cy3. The number of samples and the amount of fluorescent dye, as well as internal standard 1, were set as above. In the dark, the IBS group samples (50 µg, 5-10 µL) were labelled with 1 µL (400 pmol) of CyDye Cy3. The treatment group samples (50 µg, 5-10 µL) were labelled with 1 µL (400 pmol) of CyDyeCy5, and the pooled samples from the IBS group and treatment group (25 µg of each) were labelled with 1 µL (400 pmol) of CyDyeCy2 (as internal standard 2). An additional reverse-labelling experiment was conducted, i.e., the IBS group samples were labelled with CyDyeCy5, and the treatment group samples were labelled with CyDyeCy3. The number of samples and the amount of fluorescent dye, as well as internal standard 2, were set as above. After incubation on ice (for 30 min) in the
Screening of differentially expressed protein spots
Two-dimensional fluorescence difference gel electrophoresis (2D DIGE) was used as previously reported. Briefly, Cy3- and Cy5-labelled samples from each group (50 μg) were combined before mixing with 50 μg of Cy2-labelled internal standard. Then, an equal volume of 2 x sample buffer was added to the sample, and the total volume was increased to 450 μL with rehydration buffer. The samples were actively rehydrated and added onto 24-cm IPG strips and isoelectric focusing (IEF) was performed. Then, at room temperature, the IPG strips were incubated in equilibration buffer supplemented with 0.5% DTT for 15 min, followed by incubation in equilibration buffer supplemented with 4.5% iodoacetamide for 15 min. The IPG strips were then placed on 12% homogeneous polyacrylamide gels precast with low-fluorescence glass plates, using an Ettan DALT gel caster. Then, second dimension sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the Protein Plus system (Bio-Rad). After 2-D electrophoresis, the gels were scanned on the Typhoon 9410 scanner with Ettan DALT gel alignment guides, using emission wavelengths specific for Cy2 (480/530 nm), Cy3 (540/590 nm) and Cy5 (620/680 nm). The data from 2D DIGE were analysed with DeCyder software version 5.0. Protein spots that were differentially expressed in the control and IBS groups and in the IBS and treatment groups ([ratio] ≥ 1.4, P ≤ 0.01) were marked.

Protein identification
Two-dimensional electrophoresis was performed for protein identification as previously reported. We obtained 500-μg protein samples from the control and IBS groups separately, and then obtained additional 5000-μg protein samples from the IBS and treatment groups separately. Digestion and spot-picking were undertaken with preparative gels. The IPG strips were loaded with 1000 μg of protein, and the gels were stained with Coomassie Brilliant Blue dye. The protein spots of interest were excised and decolorized with 25 mM ammonium bicarbonate and 50% acetonitrile. After preparation of the gels, peptide mixtures were dissolved in 0.5% trifluoroacetic acid (TFA). Then, 1 μL of peptide solution was mixed with 1 μL of matrix containing 30% acetonitrile with 4-hydroxy-α-cyanocinnamic acid and 0.1% TFA before spotting onto the target plate. A 4800 Plus MALDI TOF/TOF instrument was used for MALDI-TOF mass spectrometry and tandem TOF/TOF mass spectrometry. Peptide mass maps were acquired in positive reflection mode. Combined mass and mass/mass spectra were used to interrogate rat sequences in the International Protein Index database (IPI Rat, version 3.52) using the MASCOT database search algorithms (version 2.0). If a protein score was greater than 60, the protein identification was considered successful. Functional classification and determination of the subcellular locations of the differentially expressed proteins were undertaken using the Gene Ontology software.

Western blotting
Samples were collected and lysed in lysis buffer containing a protease inhibitor cocktail. The proteins were fractionated by SDS-PAGE and electrophoretically transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% BSA for 2 h at room temperature, then incubated with different primary antibodies overnight at 4 °C, followed by incubation with secondary antibody. The software Quantity One (Bio-Rad) was used for densitometric analysis.

Statistical analysis
The data were compared by the Student’s t-test and one-way analysis of variance using SPSS version 17.0 (IBM, Armonk, NY, USA). The data are expressed as the mean ± standard deviation (x ± s). P < 0.05 was considered to be statistically significant.

RESULTS

Visceral hypersensitivity
In the present experiment, the volume of water required to obtain an AWR score of 3 in the IBS group [(0.90 ± 0.16) mL] was markedly lower than in the control group [(1.63 ± 0.17) mL] (P < 0.01), suggesting the presence of visceral hypersensitivity in the rats and successful establishment of the IBS model. After treatment with Tongxieyaofang, the volume of water required to obtain an AWR score of 3 in the treatment group was (1.23 ± 0.10) mL, which was markedly higher than in the IBS group (P < 0.01), indicating that Tongxieyaofang significantly attenuated the visceral sensitivity of rats with IBS (Figure 1A).

The interaction between gastrointestinal motility and visceral sensation is governed by the enteric and central nervous systems. The neuro-endocrine network that regulates gastrointestinal motility, secretion, and sensory function is the brain-intestinal axis. Furthermore, the function of the brain-intestinal axis is mediated by brain-gut peptides, including 5-HT, SP and VIP, which are important in regulating gastrointestinal motility and are closely related to the pathogenesis of IBS. Here, we detected the amount of 5-HT, SP and VIP in the plasma of rats in the control, IBS and Tongxieyaofang treatment (2 or 4 mL/kg) groups, respectively. As shown in Figure 1B-D, the protein levels of 5-HT, SP and VIP in the IBS group were significantly increased compared with the control group, while daily oral administration of Tongxieyaofang (2 or 4 mL/kg) decreased the high levels of 5-HT, SP and VIP in a dose-dependent manner.
In addition, the data obtained from HE examination of the rat colon showed that Tongxieyaofang at 4 mL/kg markedly attenuated the infiltration of inflammatory cells compared with the IBS group (Figure 2).

**Screening of differentially expressed proteins**
According to DeCyder 6.5 software analysis, the colonic mucosa tissue samples of rats in the IBS group and Tongxieyaofang treatment group contained a total of 1278 protein match points, of which, 61 protein spots were differentially expressed between the two groups. Among these 61 spots, 23 spots were upregulated in the treatment group, while 38 spots were downregulated (Figure 3).

**Identification and verification of differentially expressed proteins**
Among the 61 differentially expressed spots in the treatment group, five spots corresponding to three different gene products were clearly identified by matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF-MS) (Table 1). Two of these were upregulated, and one was downregulated in the treatment group. The two upregulated proteins were TAGLN and Aldh2, while the downregulated protein was CK8. To validate the changes in TAGLN, Aldh2 and CK8, western blotting was performed, followed by quantification and statistical analysis. As shown in Figure 4A-D, compared with the control group, the expression of TAGLN and Aldh2 in the IBS group was significantly decreased, while Tongxieyaofang (4 mL/kg) markedly promoted the expression of TAGLN and Aldh2. By contrast, the expression of CK8 was significantly increased in the IBS group, and Tongxieyaofang (4 mL/kg) obviously inhibited CK8 expression.

---

**Figure 1** Effect of Tongxieyaofang on IBS with visceral hypersensitivity in rats
A: volume of water required to obtain an AWR score of 3 in the control, IBS and Tongxieyaofang treatment (2 or 4 mL/kg) groups; B-D: protein levels of 5-HT, SP and VIP in plasma were determined by enzyme-linked immunosorbent assay analysis. 1: control; 2: IBS; 3: IBS + Tongxieyaofang (2 mL/kg); 4: IBS + Tongxieyaofang (4 mL/kg). The control and IBS groups were administered intragastrically with 0.9% NaCl solution (4 mL·kg⁻¹·d⁻¹) for 4 weeks. The treatment group received Tongxieyaofang decoction (2 or 4 mL·kg⁻¹·d⁻¹) intragastrically for 4 weeks. AWR: abdominal withdrawal reflex; IBS: irritable bowel syndrome; 5-HT: 5-hydroxytryptamine; SP: substance P; VIP: vasoactive intestinal peptide. Data are expressed as the mean ± standard deviation, *P < 0.01, †P < 0.05, vs control group, ‡P < 0.01, §P < 0.05, vs IBS group.

**Figure 2** Tongxieyaofang administration prevented colon damage in IBS model in rats (HE staining, ×200)
A: control group; B: IBS; C: IBS + Tongxieyaofang (2 mL/kg); D: IBS + Tongxieyaofang (4 mL/kg). The control and IBS groups were administered intragastrically with 0.9% NaCl solution (4 mL·kg⁻¹·d⁻¹) for 4 weeks. The treatment group received Tongxieyaofang decoction (2 or 4 mL·kg⁻¹·d⁻¹) intragastrically for 4 weeks. Serial sections of paraffin-embedded colon tissues stained with HE. Compared with the normal colon tissue (A), increased inflammatory cells infiltrated under the mucosa and epithelial cells presented derangement distribution in IBS model in rats (B). Tongxieyaofang at 2 and 4 mL/kg (C-D) markedly attenuated the infiltration of inflammatory cells and the integrity of colon barrier compared with the IBS group (B). IBS: irritable bowel syndrome; HE: haematoxylin and eosin.
In this study, we found that compared with the IBS group, when treated with Tongxieyaofang for 4 weeks, the rats in the treatment group experienced a significant decrease in visceral sensitivity ($P < 0.01$). In addi-
tion, by proteomic analysis, we revealed that the mechanism of action of Tongxieyaofang may involve the increased expression of TAGLN and Aldh2 and the reduced expression of CK8.

As mentioned above, there are multiple physiological factors causing the initiation and development of IBS. In addition, many psychological factors are also associated with IBS, such as anxiety. However, the pathological process of IBS remains unclear, and a better understanding of the pathology is critical for effective treatment. To date, pharmacological therapies for IBS specifically target the relief of particular symptoms. For example, for low-grade mucosal inflammation, anti-histamine drugs are prescribed (e.g., ebastine), and for increased faecal bile acids, bile acid sequestrants are prescribed (e.g., colestipol or colestyramine). In patients with severe symptoms, IBS is more likely caused by a combination of multiple factors; hence, a variety of pharmacological treatments are combined to achieve the best results. However, the majority of therapies only confer modest outcomes and more effective, safer drugs are needed.

Previous studies have reported that Tongxieyaofang could alleviate the severity of IBS. However, the mechanism underlying this effect remained unclear. Besides its ability to downregulate the expression of PAR-2 and proinflammatory cytokines, it has also been reported that Tongxieyaofang can decrease 5-HT in serum and SP, a calcitonin gene-related peptide, in the plasma, which contribute to the development of IBS. In addition, Tongxieyaofang was also supposed to exert therapeutic effects by inhibiting enteric mast cell activation, thus decreasing histamine secretion. In this study, we found that Tongxieyaofang might ameliorate the symptoms of IBS by promoting the expression of TAGLN and Aldh2 and inhibiting the expression of CK8.

TAGLN was first discovered in the smooth muscles of chickens by Lees-Miller et al and is highly expressed in smooth muscle, which might indicate a role in cell differentiation and cytoskeletal rearrangement. To date, most research about Transgelin has focused on its pro-tumorigenic effects, and the casual relationship between IBS and Transgelin has not been clearly stated. Aldh2 serves as one of the key enzymes in ethanol metabolism, catalysing the dehydrogenation of acetaldehyde and the generation of acetic acid, which enters the citric acid cycle and metabolises to CO₂ and H₂O. Here, by 2D-DIGE, MALDI-TOF-MS and Western blotting, we found that treatment with Tongxieyaofang significantly increased the expression of TAGLN and Aldh2 compared with the untreated IBS group, but further studies are needed to clarify the role of TAGLN and Aldh2 in visceral sensitivity.

CK8, a member of the cytokeratin family, plays an important role in maintaining the structure and function of the gastrointestinal epithelium. CK8-deficient mice developed severe chronic colitis and dysfunctional wa-ter-electrolyte transportation, leading to abnormal bacterial translocation. In addition, CK8 mutations downregulated immune function in human intestinal epithelial cells, which accounted for the occurrence of inflammatory bowel disease. It is also widely acknowledged that CK8 plays a crucial role in intestinal mucosal immunity. Increased CK8 expression could exacerbate intestinal mucosal inflammation, which is a key factor leading to visceral sensitivity and IBS. Our previous study also showed increased CK8 expression in the colon mucosa of rats with visceral hypersensitivity, which suggested low-grade inflammation in the colon mucosa. In this study, the expression of CK8 in rat colon mucosa was significantly reduced in the Tongxieyaofang treatment group compared with the untreated IBS group, with visceral sensitivity also decreasing dramatically. These findings indicated that Tongxieyaofang can reduce inflammation-related protein expression, which may be one mechanism by which it lowers rat visceral sensitivity.

Taken together, our findings verify the significant therapeutic effects of Tongxieyaofang on IBS. Considering its effectiveness and safety, Tongxieyaofang appears to be an attractive choice for IBS treatment. Regarding its mechanism of action, we revealed three candidate targets of Tongxieyaofang; however, further studies are needed to clarify their roles in the occurrence and treatment of IBS.

REFERENCES


