Regulating effect of TongXie-YaoFang on colonic epithelial secretion via Cl\(^-\) and HCO\(_3\)\(^-\) channel

Cheng Yang, Ying Xiong, Sheng-Sheng Zhang, Fang-Mei An, Jing Sun, Qing-Lin Zhang, Qiang Zhan

Abstract

AIM
To investigate the pharmacological effect of TongXie-YaoFang (TXYF) formula, a Chinese herbal formula, on Diarrhea-predominant irritable bowel syndrome (D-IBS) rats.

METHODS
In a neonatal maternal separation plus restraint stress (NMS + RS) model of D-IBS, male Sprague Dawley rats were randomly divided into two groups (NMS + RS group and TXYF-formula group) with no handlings were used as controls (NH group). Starting from postnatal
day 60, rats in TXYF-formula group were administered TXYF-formula (4.92 g/100 g bodyweight) orally twice a day for 14 consecutive days while NH group and NMS + RS group were given distilled water. Using short-circuit current technology, we observed 5-HT-induced changes of current across ion channels, such as cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel, epithelial Na⁺ channel (ENaC), Ca²⁺-dependent Cl⁻ channel (CACC), Na⁺-K⁺-2Cl⁻ co-transporter (NKCC), and Na⁺-HCO₃⁻ co-transporter (NBC), in the colonic epithelium of three groups after exposure to drugs and specific blockers with a Power Lab System (AD Instruments International).

RESULTS

Under basal conditions, the changes of short-circuit current (ΔIsc, µA/cm²) induced by 5-HT were similar in NH group and TXYF-formula group, and both higher than NMS + RS group (70.86 µA/cm² ± 12.32 µA/cm², 67.67 µA/cm² ± 11.68 µA/cm² vs 38.8 µA/cm² ± 7.25 µA/cm², P < 0.01, respectively). When CACC was blocked by 4,4'-disothiocyanato-stilbene-2,2'-disulfonic acid, 5-HT-induced ΔIsc was smaller in NMS + RS group than in NH group and TXYF-formula group, respectively (48.41 µA/cm² ± 13.15 µA/cm² vs 74.62 µA/cm² ± 10.73 µA/cm², 69.22 µA/cm² ± 11.7 µA/cm², P < 0.05, respectively). The similar result could be obtained when ENaC was blocked by Amiloride (44.69 µA/cm² ± 12.58 µA/cm² vs 62.05 µA/cm² ± 11.26 µA/cm², 62.11 µA/cm² ± 12.01 µA/cm², P < 0.05, respectively). However, when CFTR Cl⁻ channel was blocked by 1,1-dimethyl piperidinium chloride (DPC), 5-HT-induced ΔIsc did not significantly differ in three groups (42.28 µA/cm² ± 10.61 µA/cm² vs 51.48 µA/cm² ± 6.56 µA/cm² vs 47.75 µA/cm² ± 7.99 µA/cm², P > 0.05, respectively). The similar results could also be obtained in three groups when NBC and NKCC were respectively blocked by their blockers.

CONCLUSION

TXYF-formula can regulate the Cl⁻ and HCO₃⁻ secretion of colonic mucosa via CFTR Cl⁻ channel, Cl⁻/HCO₃⁻ exchanger, NBC and NKCC co-transporters.

Key words: Ion channel; Diarrhea-predominant irritable bowel syndrome; Colonic mucosa; Short-circuit current; TongXie-YaoFang formula

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. Abnormal ion secretion of colonic mucosal epithelial cells is recognized as one of the pathophysiological factors. In this paper, through the observation of TongXie-YaoFang (TXYF) formula, a Chinese herbal formula, to D-IBS rats obtained by neonatal maternal separation plus restraint stress. The mucosal stripping under a microscope was used for tissue preparation. Short-circuit current technology was used for testing 5-HT-induced changes in the current across ion channels of colonic epithelium. The results indicated that TXYF-formula could regulate the secretion of Cl⁻ and HCO₃⁻ in colonic mucosa via cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel, Cl⁻/HCO₃⁻ exchanger, Na⁺-HCO₃⁻ co-transporter and Na⁺-K⁺-2Cl⁻ co-transporter co-transporters.

INTRODUCTION

Diarrhoea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. Its clinical manifestations are characterized by diarrhoea and abdominal pain or discomfort in the absence of a demonstrable pathology. The diagnosis of D-IBS is based on symptom assessment and the Rome III Diagnostic Criteria[1-2]. According to an epidemiological study, D-IBS mainly affects young adults of 20-40 years old, and the quality of their lives is seriously affected[3].

The pathogenesis of D-IBS has not been fully clarified. Consequently, the usual treatment of the disease in Western medicine involves symptomatic therapy, which is unsatisfactory for patients while simultaneously increasing the use of health-care resources[4-6]. Because traditional Chinese medicine (TCM) can significantly improve patients’ symptoms and quality of life, increasing numbers of patients have begun to seek treatment with TCM[7,8].

A series of randomized, double-blind, placebo-controlled trials had shown that TongXie-YaoFang (TXYF) formula can significantly improve the clinical symptoms, such as diarrhea and abdominal pain or discomfort, of patients with D-IBS and improve the quality of their lives[9,10]. However, the specific mechanism of it has not been completely elaborated. The purpose of this paper is to observe the regulating effects of TXYF-formula on colonic epithelial secretion via relevant ion channels.

MATERIALS AND METHODS

Animals

Neonatal Sprague Dawley rats, postnatal day 1, were purchased from Vital River Laboratories Animal Technology Co., Ltd. (Beijing, China; Number of qualitative qualification: 11400700015068, 11400700019786), and kept at Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine [Number of permit: SYXK(Beijing)2009.0028]. In this study, only male

Yang C, Xiong Y, Zhang SS, An FM, Sun J, Zhang QL, Zhan Q. Regulating effect of TongXie-YaoFang on colonic epithelial secretion via Cl⁻ and HCO₃⁻ channel. World J Gastroenterol 2016; 22(48): 10584-10591 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i48/10584.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i48.10584
litters were used to eliminate the impact of estrogen and hormones on the secretory and sensory responses of the intestine to excitants\cite{11}. Then the pups were randomly assigned to one of the following two rearing conditions: (1) neonatal maternal separation plus restraint stress (NMS + RS); or (2) no handling or separation (NH).

**Neonatal maternal separation plus restraint stress** On postnatal days 2-21, the NMS + RS litters were removed from their cages and separated from their dams for 180 min each day, whereas NH pups remained in their home cages\cite{12,13}. Manipulation began at 0900 h ± 30 min each day to minimize the influence of circadian rhythms. During the 180 min period of separation, pups were removed from the nest to stand-alone compartments, where the temperature was maintained at 23 °C ± 0.5 °C in a thermally regulated facility. The compartments contained bedding of 2.5 cm wood chips and were adjacent to their home cages. The litters were returned to their home cages immediately after separation. All the rats were reared on a 12:12 h light-dark cycle (lights on at 0800 h) with access to food and water ad libitum. On day 22, sexes of the pups, including those in NH group, were distinguishable, so the females were removed and the males retained. On day 22, NMS + RS and NH rats were weaned and kept in individual cages with only 3-4 pups per cage. After weaning, pups were weighed once a week until the end of experiment. Manipulations were performed in the morning with the same measuring instrument and at the same location.

On days 50-59, NMS + RS rats were placed in transparent plastic restraint cylinders (4 cm × 4 cm × 18 cm), in which they could move forward and backward but could not turn around\cite{14}. The rats remained in the restraint cylinders for 3 h, with access to food and water ad libitum, in the morning and in the afternoon of each day. Then NMS + RS rats were divided into two groups (NMS + RS group and TXYF-formula group).

All animal care and experimental procedures were conducted according to the institutional ethical guidelines and conformed to the requirements of the Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine and the Animal Ethics Committee of Dongzhimen Hospital Affiliated with Beijing University of Chinese Medicine.

**TXYF Composition and administration** TXYF-formula consisted of the following four Traditional Chinese Herbal Medicines: Bai Zhu (Atractylodes macrocephala Koidz - Acta Horti Gothoburgensis 12(9): 310 1938)-9.75 g, Shao Yao (Paeonia lactiflora Pall. - Reise Russ. Reich. 3: 286. 1776)-46.875 g and Fang Feng (Sophoraphorisa divaricata (Turcz.) Schischk. - Fl. URSS 17: 359 1951.)-31.25 g. It was manufactured by Preparation Room for TCM of Beijing Chinese Medicine Hospital. All formula raw materials were examined according to the quality control criteria in Chinese Pharmacopeia\cite{15}.

From postnatal day 60, rats in TXYF-formula group were daily given orally administered TXYF-formula (4.92 g/100 g bodyweight) while NH group and NMS + RS group were treated with distilled water. The delivery volume in three groups was 2 mL/100 g per day, for 14 consecutive days.

**Tissue preparation** After treatment, the rats in three groups were first anesthetized abdominally with 7% chloral hydrate (35 mg/100 g bodyweight). The distal colon (6-7 cm from anus) was then quickly removed and incised longitudinally along the mesenteric border. The mucosa was fixed onto a Petri dish with silica gel in the bottom, with the lumen side down. The Petri dishes were filled with Krebs’ solution under 95% oxygen and 5% carbon dioxide. The mucosal layer was carefully separated from the submucosa, muscularis, and serosal layer with fine tweezers under a microscope. The mucosal layer was then cut into small flat sheets, with areas of about 0.5 cm², for further analysis. Two sheets could be obtained from one segment of the distal colon.

**Ussing chamber experiments** Six adjacent tissues from the distal colon of each rat were obtained and mounted in Ussing chambers. Krebs’ solution (5 mL) was injected into two small adjacent compartments with circulating 95% oxygen and 5% carbon dioxide while pH was maintained at 7.35-7.45 and temperature at 37 °C. The tissues were left to equilibrate for 60 min to allow the electrical parameters to stabilize. The voltage across tissues was then clamped to zero and the short-circuit current (Isc, μA/cm²) was measured. When the Isc baseline was smooth, 10 μmol/L indomethacin was added to basolateral side of the tissue and maintained for 10 min to block the influence of endogenous prostaglandin\cite{16,17}. The Isc value was recorded at this time. 5-HT (10 μmol/L) was then added to basolateral side and the maximum Isc recorded. The change in Isc (ΔIsc) after the addition of 5-HT was then calculated. To measure the transmembrane resistance (Rt, Ω/cm²), electrical stimulation of 1 mV was applied to both sides of the epithelium. Tissue conductance was calculated according to Ohm’s law and expressed in milliseconds per square centimeter (ms/cm²). The drugs or specific blockers were then applied to the apical or basolateral side of tissue and the ΔIsc of ion channels in colonic epithelium were calculated and recorded for further analysis.

**Reagents** Krebs’ solution of the following composition (in mol/L): 117 NaCl, 4.7 KCl, 1.2 MgCl₂, 24.8 NaHCO₃, 1.2 KH₂PO₄, 2.56 CaCl₂, and 11.1 glucose; Krebs’ solution without Cl⁻: sodium gluconate instead of NaCl, potassium gluconate instead of KCl, calcium gluconate instead of CaCl₂.
and the remaining components were the same; Krebs’ solution without HCO$_3^-$: 141.8 NaCl, 5.9 KCl, 1.2 MgSO$_4$·7 H$_2$O, 2.56 CaCl$_2$, 11.1 glucose, 10 HEPES free acid, and 5.6 Tris. Krebs’ solution without Cl$^-$ and HCO$_3^-$: sodium gluconate instead of NaCl and NaHCO$_3$, potassium gluconate instead of KCl, calcium gluconate instead of CaCl$_2$, and the remaining components were the same. 5-HT, batch number: 1001156278; Glibenclamide, batch number: 1001068037; Indomethacin, batch number: 1001087688; Bumetanide, batch number: 101016760; Amiloride, batch number: 101093389; SITS (4-acetamido-4’-isothio-cyanato-stilbene-2,2’-disulfonic), batch number: 1001208418; DPC (1,1-dimethyl piperidinium chloride), batch number: 101078880; BaCl$_2$, batch number: 1398; DIDS (4,4’-diisothiocyanato-stilbene-2,2’-disulfonic acid), batch number: 1001339605. cystic fibrosis transmembrane conductance regulator (CFTR)(inh)-172, batch number: 6311. All reagents were purchased from Sigma-Aldrich Co.

Apparatus
A multichannel voltage-current clamp (VCC MC6) was purchased from Physiologic Instruments Corporation; the Bridge amplifier (ML228), recording and analysis system for physiological data (Power Lab) was purchased from AD Instruments Corporation.

Statistical analysis
All experimental data were analyzed by SPSS 17.0 statistical software and expressed as means ± SE. The differences between groups were analyzed with a paired t test. $P < 0.05$ was considered statistically significant.

RESULTS

Basic electrophysiological properties of colonic mucosa in three groups were undifferentiated
The basic Isc did not differ significantly between three groups (21.02 μA/cm$^2$ ± 2.92 μA/cm$^2$ vs 20.29 μA/cm$^2$ ± 3.58 μA/cm$^2$ vs 20.71 μA/cm$^2$ ± 2.04 μA/cm$^2$, $n = 18$, $P > 0.05$, respectively; Figure 1A). The basic voltage in three groups had similar results (3.49 mV ± 0.54 mV vs 3.52 mV ± 0.69 mV vs 3.57 mV ± 0.62 mV, $n = 18$, $P > 0.05$, respectively; Figure 1B) as well as the basic resistance (166.8 Ω/cm$^2$ ± 20.11 Ω/cm$^2$ vs 173.66 Ω/cm$^2$ ± 16.39 Ω/cm$^2$ vs 171.94 Ω/cm$^2$ ± 19.03 Ω/cm$^2$, $n = 18$, $P > 0.05$, respectively; Figure 1C). The 5-HT-induced change of short-circuit current

![Figure 1: Comparison of basic electrophysiological properties of colonic mucosa in rats and 5-HT-induced changes of short-circuit current among three groups. A-C: The basic Isc, basic voltage and basic resistance did not differ significantly between three groups, respectively ($P > 0.05$, respectively); D: TXYF-formula significantly increased 5-HT-induced ∆Isc in neonatal maternal separation plus restraint stress (NMS + RS) rats. $P < 0.01$ vs NMS + RS group. Isc: The short-circuit current; ∆Isc: The changes of Isc; TXYF: TongXie-YaoFang; NMS: Neonatal maternal separation; RS: Restraint stress; NH: No handlings as control.](https://www.wjgnet.com/)
(ΔIsc) in NMS + RS group was respectively smaller than that in NH group and TXYF-formula group (38.8 μA/cm² ± 7.25 μA/cm² vs 70.86 μA/cm² ± 12.32 μA/cm², 67.67 μA/cm² ± 11.68 μA/cm², n = 18, P < 0.01, respectively; Figure 1D).

5-HT-induced ΔIsc after the effects of CFTR Cl⁻ channel or Na⁺-K⁺-2Cl⁻ co-transporter blocker was similar in three groups. ΔIsc was higher in TXF- formula group than in NMS + RS group after the effects of CFTR Cl⁻ channel or epithelial Na⁺ channel blocker Amiloride. DPC or Glibenclamide with final concentration 1 mmol/L were respectively added to apical side, namely left side of Ussing chamber, of colonic mucosa and equilibrated for 30 min. Then 10 μmol/L 5-HT was added to basolateral side (Equilibrated time and added concentration for 5-HT was same in the following experiment). ΔIsc induced by 5-HT were similar in TXF-group and NMS + RS group, respectively (47.75 μA/cm² ± 7.99 μA/cm² vs 42.28 μA/cm² ± 10.61 μA/cm², 57.57 μA/cm² ± 14.25 μA/cm² vs 46.78 μA/cm² ± 11.68 μA/cm², n = 8, P > 0.05, respectively; Figure 2A). The similar result was obtained when CFTR(inh)-172 was added to apical side with final concentration 100 μmol/L (45.04 μA/cm² ± 9.18 μA/cm² vs 36.2 μA/cm² ± 9.64 μA/cm², n = 8, P > 0.05; Figure 2A).

5-HT-induced ΔIsc was higher in TXF-formula group than in NMS + RS group after the effects of Ca²⁺-dependent Cl⁻ channel blocker DIDS (500 μmol/L, added to apical side) or epithelial Na⁺ channel blocker DIDS (500 μmol/L, added to apical side), respectively (69.22 μA/cm² ± 11.7 μA/cm² vs 48.41 μA/cm² ± 13.15 μA/cm², 62.11 μA/cm² ± 12.01 μA/cm² vs 44.69 μA/cm² ± 12.58 μA/cm², n = 8, P < 0.05, respectively; Figure 2B). There were no statistical differences in three groups when 100 μmol/L Na⁺-K⁺-2Cl⁻ co-transporter blocker (NKCC) blocker Bumetanide was added to basolateral (37.64 μA/cm² ± 10.57 μA/cm² vs 27.55 μA/cm² ± 10.94 μA/cm² vs 29.43 μA/cm² ± 7.66 μA/cm², n = 8, P > 0.05; Figure 2B).

There were no significant differences in three groups after the effects of K⁺ channel blocker, Na⁺-HCO₃ co-transporter blocker or Cl⁻/HCO₃⁻ exchanger inhibitor on 5-HT-induced ΔIsc. ΔIsc induced by 5-HT were similar in three groups when 5 mmol/L K⁺ channel blocker BaCl₂ or 100 μmol/L Cl⁻/HCO₃⁻ exchanger inhibitor SITS was respectively added to basolateral side of colonic mucosa (25.63 μA/cm² ± 13.69 μA/cm² vs 13.92 μA/cm² ± 8.16 μA/cm² vs 17.03 μA/cm² ± 9.04 μA/cm², 49.92 μA/cm² ± 11.66 μA/cm² vs 40.41 μA/cm² ± 14.26 μA/cm² vs 47.7 μA/cm² ± 11.43 μA/cm², n = 8, P > 0.05, respectively; Figure 3). There was also no statistic difference in three groups after the effects of 200 μmol/L Na⁺-HCO₃ co-transporter (NBC) blocker DIDS (500 μmol/L, added to basolateral side) (12.27 μA/cm² ± 3.6 μA/cm² vs 10.74 μA/cm² ± 2.99 μA/cm² vs 11.88 μA/cm² ± 3.51 μA/cm², n = 8, P > 0.05; Figure 3).

5-HT-induced ΔIsc was higher in TXF-formula group than in NMS + RS group when Na⁺ in apical side of colonic mucosa was taken out. This phenomenon disappeared while Cl⁻ and HCO₃⁻ were taken out altogether or respectively. When Na⁺ applied to apical side of colonic mucosa was substituted with sodium gluconate, 5-HT-induced ΔIsc was higher in TXF-formula groups than in NMS + RS group (56.86 μA/cm² ± 11.2 μA/cm² vs 39.14 μA/cm² ± 10.83 μA/cm², n = 8, P < 0.05; Figure 4A). However, when basolateral side Na⁺ was substituted with sodium gluconate, ΔIsc was similar in three groups (0.87 μA/cm² ± 0.33 μA/cm² vs 1.15 μA/cm² ± 0.5 μA/cm² vs 1.01 μA/cm² ± 0.49 μA/cm², n = 8, P > 0.05; Figure 4B).
The etiology and pathogenesis of D-IBS are complex, and its pathophysiological changes predominantly include dynamic gastrointestinal disorder and visceral sensory sensitivity. In TCM, D-IBS is classified as diarrhea or abdominal pain according to its clinical manifestations\(^\text{[18]}\). Previous studies had shown that TXYF-formula had an inhibitory effect on bowel movement and reduced intestinal peristalsis by regulating secretion of Cl\(^-\) and HCO\(_3\)\(^-\) in rats treated with DIDS. Therefore, the regulatory effect of TXYF-formula is achieved by regulating secretion of Cl\(^-\) and HCO\(_3\)\(^-\) as that observed in NH rats.

We also found that TXYF-formula alters HCO\(_3\)\(^-\) secretion. When extracellular HCO\(_3\)\(^-\) was removed or NBC was inhibited, 5-HT-induced ∆Isc did not differ between NMS + RS group and TXYF-formula group. The same result was obtained when extracellular Cl\(^-\) and HCO\(_3\)\(^-\) were both removed or when Cl\(^-\)/HCO\(_3\)\(^-\) exchange was interrupted. Thus, the therapeutic effect of TXYF-formula is achieved by regulating the secretion of both Cl\(^-\) and HCO\(_3\)\(^-\). Moreover, when Na\(^+\) on apical side of membrane was removed or a Na\(^+\) channel blocker was added, ∆Isc still differed between NMS + RS group and TXYF-formula group; and the differences between NMS + RS and NH rats did not disappear. Therefore, the relationship between therapeutic effects of TXYF-formula on Na\(^+\) transport and D-IBS is not close. Nevertheless, after Na\(^+\) in basilar membrane was removed, the electrical activity across epithelium did not differ between TXYF-formula group and NMS + RS group. Therefore, it can be seen that the regulatory effects of TXYF-formula on Cl\(^-\) and HCO\(_3\)\(^-\) secretion depends on Na\(^+\) in basilar membrane.

There are two main types of Cl\(^-\) channels on apical side of epithelial cells: CFTR and CACC. CFTR is a cAMP-dependent, PKA-activated Cl\(^-\) channel, and is sensitive to DPC and Glibenclamide\(^\text{[23,24]}\). After CFTR Cl\(^-\) channel was blocked by DPC or Glibenclamide, the electrical activity across epithelium induced by 5-HT did not differ obviously between TXYF-formula group and NMS + RS group. However, the electrical activity in two groups differed markedly after the application of CACC blocker DIDS. Therefore, the regulatory effect of DIDS on mucosae of NMS + RS rats was weaker than those in NH rats or TXYF-formula-treated rats in this study. This may be related to increased 5-HT and 5-HT-receptor levels in NMS + RS rats. When 5-HT was added to colonic mucosa from NMS + RS rats, the electrical activities across epithelium declined. The reaction to 5-HT in colonic mucosae of rats was restored by treatment with TXYF-formula, to a level almost the same as that observed in NH rats. This demonstrates that TXYF-formula has specific therapeutic effects on D-IBS.
of TXYF-formula on electrical activity across epithelia of D-IBS rats is associated with CFTR Cl$^-$ channel, and has nothing to do with CACC channel.

Our results, combined with preliminary studies\(^{25,26}\) about secretory mechanisms of epithelial anions in colonic mucosa, allow the following conclusions to be drawn. The regulating effects of TXYF-formula on D-IBS involves the secretion of Cl$^-$ and HCO$_3^-$ in colonic mucosa via CFTR Cl$^-$ channel, Cl$^-$/HCO$_3^-$ exchanger, and NBC and NKCC co-transporters.

**COMMENTS**

**Background**

Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic functional gastrointestinal disease. The pathogenesis of it has not been thoroughly elucidated while colonic abnormal secretory is recognized as one of the pathophysiological factors. The usual treatment in Western medicine, mainly involves symptomatic therapy, is unsatisfactory for patients while simultaneously increasing the use of health-care resources. Traditional Chinese medicine (TCM) can obviously alleviate patients’ clinical symptoms, increasing numbers of them have begun to seek treatment with TCM. A lot of researches have shown that TongXie-YaoFang formula (TXYF-formula), a Chinese herbal formula, can significantly improve D-IBS patients’ clinical symptoms and enhance their quality of lives.

**Research frontiers**

TXYF-formula is a Traditional Chinese classical prescription for clinical treatment on D-IBS. The research hotspot is its effect on colonic abnormal secretory via correlational ion channels, visceral sensitivity and colon movement.

**Innovations and breakthroughs**

Previous clinical and experimental studies had only shown that TXYF-formula can relieve diarrhea of patients with D-IBS. Its correlational effects might be realized by influencing secretion of colon. However, the specific mechanism and correlative ions are unclear. In this study, the mucosal stripping under a microscope was used for tissue preparation. And short-circuit current technology was applied to observe 5-HT-induced changes in current across ion channels of colonic epithelium so as to reveal the prescription effect of TXYF-formula ion transport in colon.

**Applications**

The key results of this study showed that TXYF-formula can regulate the secretion of Cl$^-$ and HCO$_3^-$ in colonic mucosa of D-IBS rats. And this may be related to cystic fibrosis transmembrane conductance regulator (CFTR) Cl$^-$ channel, Cl$^-$/HCO$_3^-$ exchanger, and Na$^+-$HCO$_3^-$ co-transporter (NBC) and Na$^+$-K$^-$-2Cl$^-$ co-transporter (NKCC) co-transporters.
Terminology
D-IBS, as a clinically common functional gastrointestinal disease, is closely related to early adverse life events. It seriously affects patient’s quality of jobs and lives. TXTF-formula can regulate the Cl- and HCO₃⁻ secretion of colonic mucosa via CFTR Cl channel, Cl/HCO₃⁻ exchanger, NBC and NKCC co-transporters.

Peer-review
Well written, quite meticulous methodology, nicely executed study.

REFERENCES
1. Ford AC, Berek P, Morgan DG, Bolino C, Pintos-Sanchez MJ, Moayyedi P. Validation of the Rome III criteria for the diagnosis of irritable bowel syndrome in secondary care. Gastroenterology 2013; 145: 1262-70.e1 [PMID: 23994201 DOI: 10.1053/j.gastro .2013.08.048]
2. Shalaby SA, Sayed MM, Ibrahim WA, Abdelhamak SM, Rushdy M. The prevalence of coeliac disease in patients fulfilling Rome III criteria for irritable bowel syndrome. Arab J Gastroenterol 2016; 17: 73-77 [PMID: 27349575 DOI: 10.1016/j.ajge.2016.05.003]
3. Marquis P, Lasch KE, Delgado-Herrera L, Kohlari S, Lembro A, Lademacher C, Spears G, Nishida A, Tesler WL, Piault E, Rosa K, Zeiber B. Qualitative development of a patient-reported outcome symptom measure in diarrhea-predominant irritable bowel syndrome. Clin Transl Gastroenterol 2014; 5: e59 [PMID: 24964944 DOI: 10.1038/ctg.2014.7]
4. Nee J, Zakari M, Lembro AJ. Current and emerging drug options in the treatment of diarrhea predominant irritable bowel syndrome. Expert Opin Pharmacother 2015; 16: 2781-2792 [PMID: 26558923 DOI: 10.15171/14656566]
5. Lacy BE. Diagnosis and treatment of diarrhea-predominant irritable bowel syndrome. Int J Gen Med 2016; 9: 7-17 [PMID: 26929659 DOI: 10.2147/IGM]
6. Chiba T, Yamamoto K, Sato S, Suzuki K. Long-term efficacy and safety of ramucirumab in the treatment of diarrhea-predominant irritable bowel syndrome. Clin Exp Gastroenterol 2013; 6: 123-128 [PMID: 23922505 DOI: 10.2147/CEG.S32721]
7. Teschke R, Wolf A, Frenzel C, Hichoff A, Schulze J. Herbal traditional Chinese medicine and its evidence base in gastrointestinal disorders. World J Gastroenterol 2015; 21: 4466-4490 [PMID: 25914456 DOI: 10.3748/wjg.v21.i15.4446]
8. Xiao Y, Liu Y, Huang S, Sun X, Tang Y, Cheng J, Wang T, Li F, Kuang Y, Luo R, Zhao X. The efficacy of Shugan Jianpi Zhixie granules in treating diarrhea-predominant irritable bowel syndrome. Chin J Integr Med 2009; 15: 216-219 [PMID: 19568715 DOI: 10.1007/s11655-009-0216-7]
9. Lai LJ, Han YC, Hui Zhang, Zhu XW, Han XM. The timeliness of Tongxie-YaoFang on patients with D-IBS about improving the clinical symptoms. Shiyong Zhongyi Jiehe Linchuang 2013; 13: 27-28 [DOI: 10.3969/j.issn.1671-4040.2013.02.017]
10. Ren TH, Wu J, Yew D, Ziao E, Lao L, Leung WK, Berman B, Hu PJ, Sang JJ. Effects of neonatal maternal separation on neurochemical and sensory response to colonic distension in a rat model of irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2007; 292: G849-G856 [PMID: 17110521 DOI: 10.1152/ajpgi.00400.2006]
11. Gareau MG, Jury J, Perdue MH. Neonatal maternal separation of rat pups results in abnormal cholinergic regulation of epithelial permeability. Am J Physiol Gastrointest Liver Physiol 2007; 293: G198-G203 [PMID: 17510196 DOI: 10.1152/ajpgi.00392.2006]
12. Oines E, Mursion R, Mrdal J, Grunil J, Milde AM. Neonatal maternal separation in male rats increases intestinal permeability and affects behavior after chronic social stress. Physiol Behav 2012; 105: 1058-1066 [PMID: 22155491 DOI: 10.1016/j.physbeh.2011.11.024]
13. Irles C, Nava-Kopp AT, Morán J, Zhang L. Neonatal maternal separation up-regulates protein signalling for cell survival in rat hypothalamus. Stress 2014; 17: 275-284 [PMID: 24730533 DOI: 10.3109/10253890.2014.913017]
14. Pharmacopoeia Commission of the Ministry of Public Health of PRC. Chinese Pharmacopoeia: Chemical Industry Press, 2005
15. Fung SY, Chernova J, Hastings M, Lam C, Marciani L, Singh G, Henry A, Hall I, Whorvell P, Spiller R. A randomised trial of ondansetron for the treatment of irritable bowel syndrome with diarrhea. Gut 2014; 63: 1617-1625 [PMID: 24334242 DOI: 10.1136/gutjnl-2013-305989]
16. Feng XY, Li Y, Li LS, Li XF, Zhang LF, Zhang XL, Fan RF, Song J, Hong F, Zhang Y, Zha JX. Dopamine D1 receptors mediate dopamine-induced duodenal epithelial ion transport in rats. Transl Res 2013; 161: 486-494 [PMID: 23276732 DOI: 10.1016/j.trsl.2012.12.002]
17. Li L, Zhang SS. The mechanism research status in Irritable bowel syndrome with traditional Chinese medicine. Zhongguo Zhongxiyi Jiehe Zazhi 2012; 20: 466-470
18. Rahimi R, Abdollahi M. Herbal medicines for the management of irritable bowel syndrome: a comprehensive review. World J Gastroenterol 2012; 18: 589-600 [PMID: 22363129 DOI: 10.3748/wjg.v18.i7.589]
19. Yang C, Zhang SS, Li XL, Wang ZF, Zhao LQ. Inhibitory effect of TongXie-YaoFang formula on colonic contraction in rats. World J Gastroenterol 2015; 21: 2912-2917 [PMID: 25780288 DOI: 10.3748/wjg.v21.i10.2912]
20. Hu XG, Liao SL, Wang YF, Gong MJ, Wang Man, Liu SS, Han Bin. Effect of Radix Saposhnikoviae in Tongxie Yaofang on PAR2 mRNA Expression and Inflammatory Mediators in the Colon of Rats with Postinfection Irritable Bowel Syndrome. Zhongyao Linchuang Yauoile 2013; 24: 5-9
21. Fujita T, Karaki S, Tateoka T, Kuvahara A. Desacetyl bisacodyl-induced epithelial Cl(-) secretion in rat colon and rectum. Biomed Res 2016; 37: 13-20 [PMID: 26912136 DOI: 10.2220/biomedres.37.13]
22. Ao M, Sarathy J, Domingue J, Alrefai WA, Rao MC. Chenede- oxycyclic acid stimulates Cl(-) secretion via cAMP signaling and increases cystic fibrosis transmembrane conductance regulator phosphorylation in T84 cells. Am J Physiol Cell Physiol 2013; 305: C447-C456 [PMID: 23761628 DOI: 10.1152/ajpcell.00416.2012]
23. Hou Y, Guan X, Yang Z, Li C. Emerging role of cystic fibrosis transmembrane conductance regulator - an epithelial chloride channel in gastrointestinal cancers. World J Gastroenterol Oncol 2016; 8: 282-288 [PMID: 26989463 DOI: 10.4253/wjgo.v8.i3.282]
24. Dürnagel S, Falkenburger BH, Gründler S. High Ca(2+) permeability of a peptide-gated DEG/ENaC from Hydra. J Gen Physiol 2012; 140: 391-402 [PMID: 23008433 DOI: 10.1085/jgp.201210798]
25. Cremon C, Carini G, Wang B, Vasina V, Cagliandro RF, De Giorgio R, Stanghellini V, Grundy D, Tonini M, De Ponti F, Corinaldesi R, Barbara G. Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. Am J Gastroenterol 2011; 106: 1290-1298 [PMID: 21427712 DOI: 10.1038/ajg.2011.86]

P-Reviewer: Garg P   S-Reviewer: Qi Y   L-Reviewer: A   E-Editor: Liu WX
