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Original Research Article

Tong-Xie-Yao-Fang improves chronic intestinal fibrosis in mice with colitis through CRH-R2-mediated IL-6/STAT3 signaling pathway

Shiyi Wang[1]*, Xudong Zhang[1], Xiaojian Wang[2], Yihong Fan[3]
[1]Department of Gastroenterology, Ningbo Municipal Hospital of TCM Affiliated Hospital of Zhejiang Chinese Medical University, No. 819 North Liyuan Road, Ningbo, Zhejiang 315000, [2]Department of Gastroenterology, Lihuili Hospital of Ningbo Medical Center, No. 57 Xingning Road, Ningbo, Zhejiang 315040, [3]Department of Gastroenterology, The First Affiliated Hospital of Zhejiang Chinese Medical University, No. 54 Youdian Road, Hangzhou, Zhejiang 310006, China

For correspondence:* ***Email:* *wangshiyi2@126.com;* ***Tel:*** *+86-0571-87089085*

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Abstract

Purpose: *To investigate the potential mechanism of action of Tong-Xie-Yao-Fang (TXYF) on inflammation-related intestinal fibrosis in mice. In addition, to examine dextran sodium sulfate (DSS) induction and the stimulation of human intestinal fibroblasts (CCD-18Co) by transforming growth factor-β1 (TGF-β1).*

Methods: *Chronic colitis in C57BL/6 mice was induced by administering 2 % DSS and TXYF extract (11.2 g/kg per day). An intestinal fibrosis cell model was constructed by stimulating CCD-18Co cells with TGF-β1 (10 ng/mL). The status and weight loss of colitis of the mice was assessed by Disease Activity Index (DAI) while colonic tissue injury and fibrosis were assessed using hematoxylin-eosin (H&E) staining and Masson's trichrome staining. Relative expression levels of corticotrophin-releasing hormone receptor 2 (CRH-R2) and Interleukin 6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) in colon tissues and CCD-18Co cells were determined using Western blot. A cell model of intestinal fibrosis was constructed by stimulating CCD-18Co cells with TGF-β1 (10 ng/mL). At the same time, the cells were subjected to the intervention of TXYF drug-containing serum (TXYF-DS).*

Results: *TXYF significantly improve the pathological changes, weight loss, and DAI score of colon tissue in DSS induced colitis mice ($p < 0.05$). The expression levels of IL-6 and p-STAT3 were significantly reduced, while the expression level of CRH-R2 was upregulated in the mouse colon tissue ($p < 0.05$). At the cellular level, TXYF-DS intervention significantly reduced cell mortality and mitigated the degree of cell fibrosis ($p < 0.05$). Furthermore, the expression level of CRH-R2 was up-regulated while that of IL-6 and p-STAT3 were down-regulated.*

Conclusion: *TXYF ameliorates chronic intestinal fibrosis in colitis-afflicted mice by restricting IL-6/STAT3 signaling pathway whilst upregulating CRH-R2 expression.*

Keywords: *Colitis, Chronic intestinal fibrosis, Tong-Xie-Yao-Fang, CRH-R2, IL-6/STAT3 pathway*

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INTRODUCTION

Ulcerative colitis (UC) is a common inflammatory disorder of the gastrointestinal tract that causes stomach ache and diarrhea, and may further lead to intestinal constriction and fibrosis [1]. For several decades, the global incidence rate of UC has been on the rise, thus turning into a natural

disease of the alimentary system. It has a major influence on the patient's health and quality of life as well as elevates the medical burden of the society [2].

Intestinal fibrosis is a naturally occurring condition associated with UC, mostly caused by the inflammation-induced damage to the intestinal mucosa. Fibrosis is caused by excessive sedimentation of the extracellular matrix (ECM), which is produced by activated myofibroblasts and modulated by pro-fibrotic and anti-fibrotic factors [3]. Fibrosis is a natural physiological repairing process; however, healing process becomes harmful if it lasts for a long time due to toxic substances present throughout the process, thus leading to inflammation and production of growth factors, proteolytic enzymes and fibrotic cytokines that cause fibrosis. This process involves activation of non-immune and immune cells, macrophages, and T cells, and their interaction is crucial for the progressive remodeling and destruction of tissues [4].

Currently, there are relatively limited treatment options for intestinal fibrosis caused chronic inflammation. In most cases, surgical excision of the affected tissue is considered the primary treatment approach. TXYF is an authentic and scientifically recognized traditional Chinese herbal recipe used to treat inflammatory bowel disease. TXYF is composed of four medicinal herbs: white rhizoma atractylodes, paeoniae radix alba, tangerine peel and sand ginseng. It has been reported to have immunomodulatory and anti-inflammatory functions. Numerous studies suggest that TXYF regulates intestinal flora, cell signal transduction and brain-gut axis in UC. Additionally, it may enhance targeting of bone marrow mesenchymal stem cells (BMMSCs) to the colon mucosa, and promote healing of the colon mucosal barrier [5].

Studies based on modern network pharmacology has confirmed that TXYF plays a multi-link, multi-pathway and multi-target influence in the healing of inflammatory bowel disease [6]. Previous studies have demonstrated that TXYF decrease colon 5-HT through the GCN2/PERK eIF2α-ATF4 signaling pathway, inhibit the Notch and NF-κB signaling pathways [12], as well as relieve symptoms of diarrheal irritable bowel syndrome (IBS-D) in rats by regulating the microbiota levels in the intestinal flora [7]. However, there are ongoing studies investigating the impact of TXYF on the chronic intestinal fibrosis caused by colitis. As such, the goal of this current investigation was to examine the therapeutic efficacy of TXYF on the chronic intestinal fibrosis in colitis and determine its mechanism against fibrosis.

EXPERIMENTAL

Preparation of TXYF

TXYF was prepared by combining white *Rhizoma atractylodes*, *Paeoniae radix* alba, tangerine peel and sand ginseng at a ratio of 15:12:6:10 [8]. Subsequently, the herb-medicines mixture was immersed in distilled water for 1 hour and boiled for 30 min. The boiled mixture was filtered and then boiled for 30 min. The liquid obtained from the previous boiling and filtration processes were mixed at concentrated of 1:1 (100 % consistency) ratio and preserved at 4 ℃ for future use.

Animal models and drug therapy

A total of 30 8-week-old male C57BL/6 mice weighing 20 - 25 g were provided by Zhejiang Academy of Medical Sciences, China and kept in the SPF animal house of the Laboratory Animal Center of Hangzhou Medical College (temperature: 22-26 ℃, humidity: 45 % - 55 %, 12-hour light system). The mice were provided with adaptive feeding for a duration of one week before the experiment, allowing them unrestricted access to water and food. The animal experiments undertaken adhered to the National Institutes of Health Laboratory Animal Care and Use Guidelines [9] and were authorized by the Ethics Committee of Zhejiang Center of Laboratory Animals (no. ZJCLA-IACUC-20020105).

The C57BL/6 mice were stochastically categorized into 3 groups: control group (n = 10), Dextran sulfate sodium (DSS) (item number 42867, MP Biomedical, OH, USA) group (n = 10) and DSS + TXYF group (n = 10). The concentration of DSS was 2 %, and the TXYF water extract dose was 11.2 g/kg per day (corresponding to the usual dosage for adults). The mice were administered 2 % DSS-contained water instead of sterile water for 3 cycles to induce chronic colitis, with 7 days of 2 % DSS-contained water + 14 days of sterile water for each cycle. Meanwhile, mice in the DSS + TXYF group received TXYF by gavage for 3 cycles, and the control group mice were administered sterile water. After 3 cycles, the experimental mice were euthanized by cervical dislocation of their joints and their distal colon was taken for subsequent experiments.

Disease activity index (DAI)

Intestinal DAI was examined in relation to the degree of weight loss, diarrhea and bleeding from the colon. There were five levels of weight

loss viz: 0 (none), 1 (1 - 5 %), 2 (5 - 10 %), 3 (10 - 20 %), 4 (> 20 %). In the event of consistent stool (diarrhea), classification was done as follows: presence of food particles with normal shape (0), semi-formed stool (1), loose stool (2), liquid stool (3), diarrhea (4). Grade of rectal bleeding included: no blood (0), little blood (1), mild blood (2), obvious blood (3), severe bleeding (4). Disease Activity Index (DAI) was calculated using Eq 1.

DAI = (diarrhea score) + (weight loss score) + (proctorrhagia score)/3 …….. (1)

Hematoxylin-eosin (H&E) staining

The colon organization of mice were treated with 4 % paraformaldehyde (4 ℃ overnight), then embedded in paraffin and sliced into 4 μm thick slices. After de-waxing, standard H&E staining was performed. Histological changes of the prepared sections were examined under a microscope, then the integrity of the intestinal mucosal barrier, mucosal layer, and inflammatory cell infiltration were evaluated. 3 samples from each group were selected, with 4 areas chosen for each sample, and observed independently by 3 experimenters.

Masson staining

Masson Tri-color Dyeing Kit (item no. BP-DL021, Senbega Biotechnology Co. Ltd, Nanjing, China) was used for determination. The paraffin sections were dewaxed and dehydrated, stained with Weigert iron hematoxylin solution for 10 min, differentiated by acid ethanol for 15 sec and then fully washed and dripped with 0.1 – 1 % lithium carbonate for 5 min to increase the degree of bluing. Thereafter, the slices were stained with Ponceau S Staining Solution for 10 min, washed with 2 % glacial acetic acid for 1 minute and differentiated with 1 % phosphomolybdate water solution for 5 min. After washing with 2 % glacial acetic acid for 1 minute, the sections were stained with aniline blue for 2 min, after which they were rinsed with 0.2 % glacial acetic acid, dehydrated with gradient ethanol, subjected to vitrification with xylene, sealed with neutral gum and examined under a microscope.

Cell models and drug intervention

The Human colon fibroblast CCD-18Co (item number CL-0591, Poonosai Life Science and Technology Co., LTD, Wuhan, China) were cultured using MEM (containing NEAA + 10 % FBS + 1 % P/S) medium (item number CM-0591, Poonosai Life Science and Technology Co., Ltd, Wuhan, China) in a 37 ℃ incubator with 5 % CO_2 and humidity ≥ 90 %. CCD-18Co cells were activated with TGF-β1 at a concentration of 10 ng/mL (item number HY-P7118, MedChemExpress, New Jersey, USA) for 12 h to establish intestinal fibrosis cell model. The Sprague-Dawley (SD) rats were administered TXYF 22.4 g/kg (4 times of the adult dosage) twice a day for three consecutive days and euthanatized 1 hour after the last administration. Blood was taken from the abdominal aorta, centrifuged at 2500 rpm for 10 min for aseptic separation of serum, inactivated at 56 ℃ for 30 min and filtered with the 0.22 μm microporous membrane to discard bacteria and to prepare TXYF drug-containing serum (TXYF-Ds). siRNA was utilized to knock down CRH-R2 in the cells. After the model establishment, the CCD-18Co cells were cultured with TXYF-Ds and stained with Trypan blue for the determination of cell viability.

Trypan blue staining

Adherent cells were digested with trypsin, centrifuged at 1000 rpm for 10 min. The supernatant was discarded then 1mL of culture medium added, and the cells gently dispersed with a pipette to prepare a cell suspension. The cells were dyed with 0.04 % trypan blue solution (item number T8154, Sigma Aldrich Trading Co. Ltd, Shanghai, China) for 5 min, and then transferred to a cell counting plate. Lastly, the cells were observed under a microscope for cell count and cell viability analysis.

Western blot

An equal amount of protein was resolved on a 12.5 % SDS-PAGE gel and then transferred onto the PVDF membrane. Subsequently, the samples were blocked in 5 % BSA buffer solution for 2 h and the corresponding primary antibodies (CRH-R2 (1:1000, item number ab236982, Abbot Anti-Trade Co. Ltd, Shanghai, China), IL-6 (1:1000, mouse, item number 12912T; 1:1000, human, item number 12153S, Cell Signaling Technology, Danvers, Colorado, USA), p-STAT3 (1:1000, item number 9145T, Cell Signaling Technology, Danvers, Colorado, USA), STAT3 (1:1000, item number 9139T, Cell Signaling Technology, Danvers, Colorado, USA), Collagen I (1:1000, item number 19245T, Cell Signaling Technology, Danvers, Colorado, USA), α-SMA (1:1000, item number ab244177, Abcam, Cambridge, UK) and TIMP1 (1:1000, item number 8946S, Cell Signaling Technology, Danvers, Colorado, USA)) were added then incubated overnight at 4 ℃. The PVDF membrane was rinsed thrice with TBST and incubated at room temperature with the

secondary antibody for 2 h. The protein bands were visualized using Immobilon Western HRP substrate and normalized to GAPDH.

Data analysis

Data was expressed as mean ± standard deviation (SD) based on a minimum of 3 duplicates. *T*-test and two-factor analysis of variance was adopted for inter-group comparison. $P < 0.05$ indicated statistically significant difference. Data analysis and mapping were done using GraphPad Prism software.

RESULTS

TXYF significantly alleviates disease symptoms and histopathological changes

Compared to the control group, the DSS-induced model mice demonstrated notable weight loss ($p < 0.05$; Figure 1 A), as well as significant occurrence of diarrhea and colic bleeding. After TXYF treatment, the aforementioned symptoms significantly abated and the DAI score of the mice was significantly reduced ($p < 0.05$; Figure 1 B). Additionally, it was also found that DSS caused severe intestinal inflammation and fibrosis. As shown in Figure 1 C, DSS caused severe damage to the epithelial cells of the colon, congestion and edema in the mucosa and submucosa, accompanied by dense inflammatory cell infiltration, mucosal ulcer, crypts and goblet cells disappearance and significant fibrosis of the colon tissues (Figure 1 D). However, TXYF treatment significantly reversed the colonic damage and fibrosis in DSS-induced model mice (Figure 1 C and D), suggesting that TXYF alleviates the illness symptoms and histopathological alterations in mice with chronic colitis.

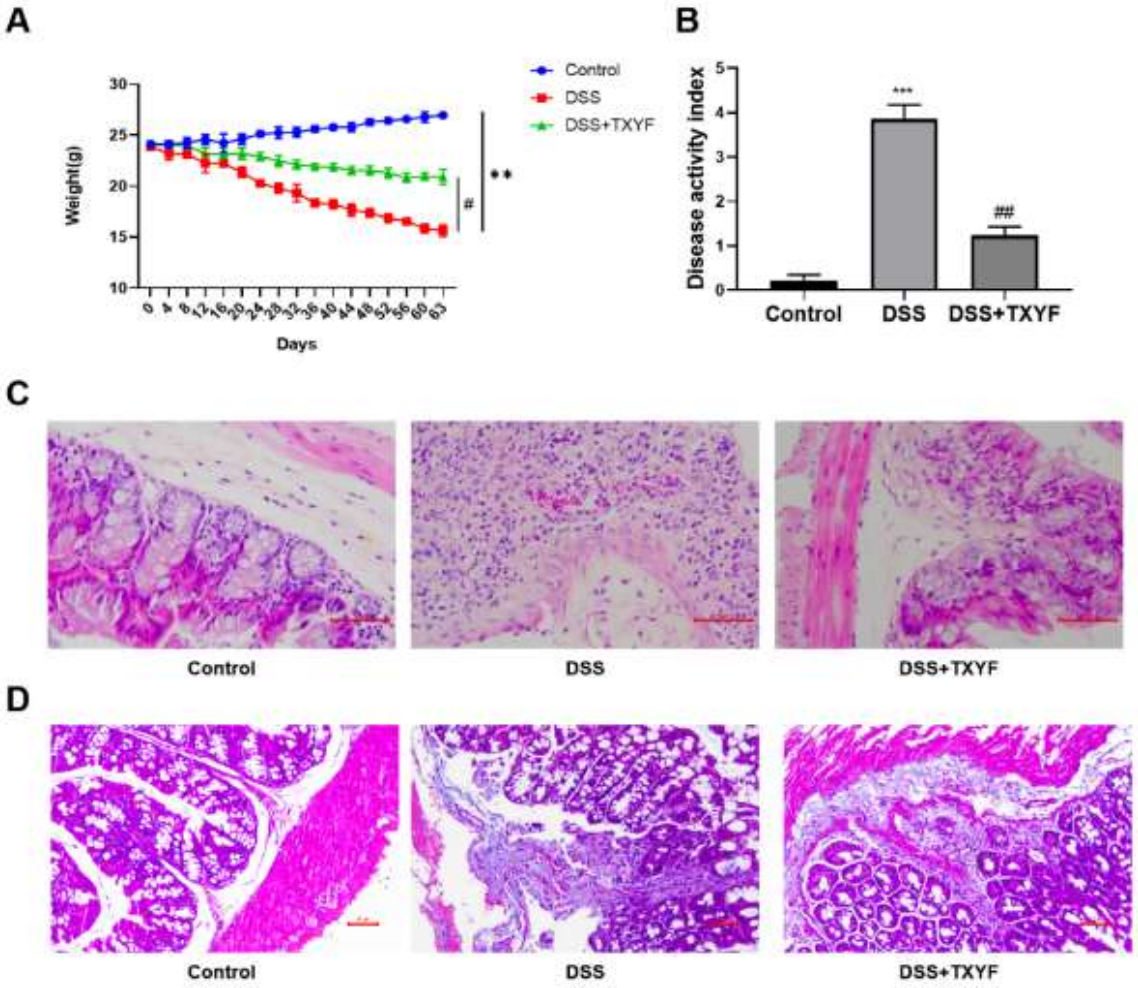

Figure 1: TXYF significantly reduces the illness symptoms and histopathological alterations of chronic colitis in mice. (A) Statistical analysis of the body weight of the experimental mice; (B) Assessment of the intestinal DAI in relation to the weight loss, diarrhea and colonic bleeding of the mice; (C) Representative image of mice large intestine tissue after H&E staining; (D) Representative image of mouse colon tissue after Masson staining. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. DSS group

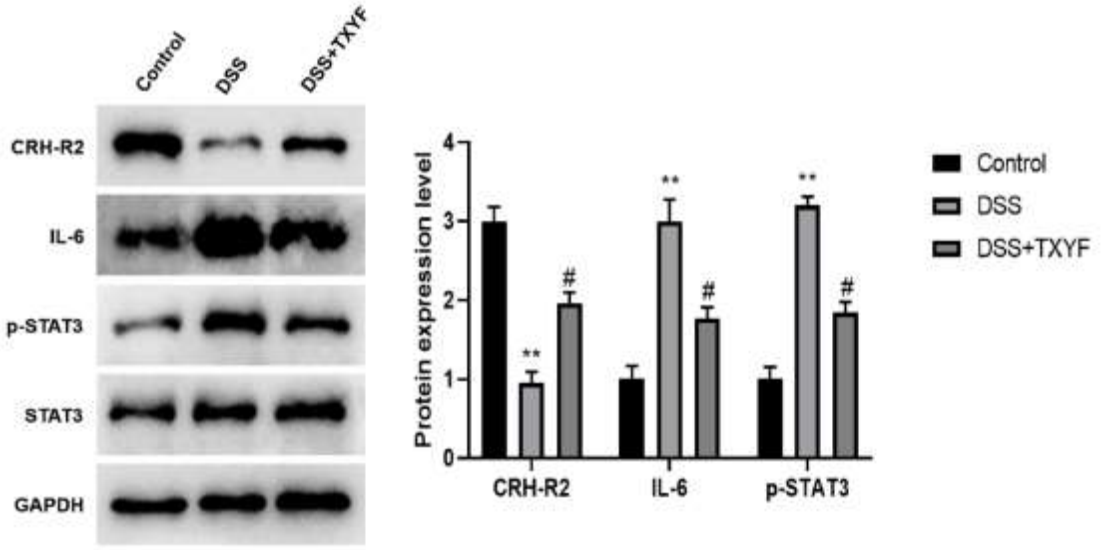

Figure 2: Changes of CRH-R2 expression and IL-6/STAT3 pathway-related protein phosphorylation in the gut tissues of mice with chronic colitis (Western Blot). $^{\#}P < 0.05$ vs. DSS group, $^{**}P < 0.01$ vs. control group

TXYF promotes expression of CRH-R2 and inhibits phosphorylation of IL-6/STAT3 pathway-related proteins

To determine the changes of phosphorylation of CRH-R2 and IL-6/STAT3 pathway-related proteins in colonic tissues of mice with colitis, the expression levels of the related protein level was assessed using Western blot. The findings demonstrated that in comparison to the control group, the CRH-R2 level and phosphorylation levels of IL-6 and STAT3 in the large intestine tissue of mice in the DSS group were substantially reduced (Figure 2). Compared to the DSS group, the CRH-R2 level in TXYF group was substantially increased, along with a notable decrease in the IL-6 content and phosphorylation level of STAT3. This suggests that TXYF significantly inhibited the production of IL-6 and phosphorylation of STAT3 in chronic colitis mice and reversed the decrease of CRH-R2 level caused by exposure to DSS.

TXYF improves the cell fibrosis of TGF-β1-excited CCD-18Co cells

To determine the impact of TXYF on cell fibrosis, CCD-18Co cells were activated with TGF-β1 (10 ng/mL) for 12 h to establish a cell model of intestinal fibrosis. After model establishment, cells were cultured with TXYF-Ds and stained with Trypan blue for the determination of cell viability. The levels of fibrosis marker proteins α-SMA, Collagen I, TIMP1 and CRH-R2 were determined by Western blot. After TGF-β1 stimulation, a notable increase was seen in the number of cell death (Figure 3A) and a decrease was shown in the cell viability. Besides, the levels of fibrosis marker proteins α-SMA, CollagenI, TIMP1 were significantly elevated (Figure 3B) and the degree of fibrosis was enhanced. Similarly, the same trend was revealed in the level of CRH-R2 as that in the colitis mouse model. Compared to the TGF-β1+NC-DS group, the number of cell death in the TGF-β1+ TxyF-DS group was substantially decreased, the content of CRH-R2 was raised, and the degree of cell fibrosis was reduced. These findings suggest that TXYF effectively improves TGF-β1 induced cell fibrosis.

TXYF improves cell fibrosis through CRH-R2

Based on the experimental results, TXYF demonstrated a significant enhancement in fibrosis at both animal and cellular levels. CRH-R2 was revealed to be under-expressed in both the mouse models and the cellular models of colitis, however, the declining trend of CRH-R2 was reversed after TXYF treatment. To verify whether TXYF improves fibrosis by regulating CRH-R2 levels, si-RNA was used to knock down CRH-R2 in the cell model after drug treatment, to determine changes in cell viability and fibrosis levels. The Western blot results demonstrated that CRH-R2 could be effectively knocked down in cells, and a significant increase in cell death and a decreased level of cell viability were observed after CRH-R2 knockdown (Figure 4 A). Similarly, there was an elevation in the level of fibrosis marker protein (Figure 4 B), indicating that the level of fibrosis showed an opposite trend to the expression of CRH-R2. Therefore, these results indicate that TXYF enhances cell fibrosis by regulating CRH-R2 expression.

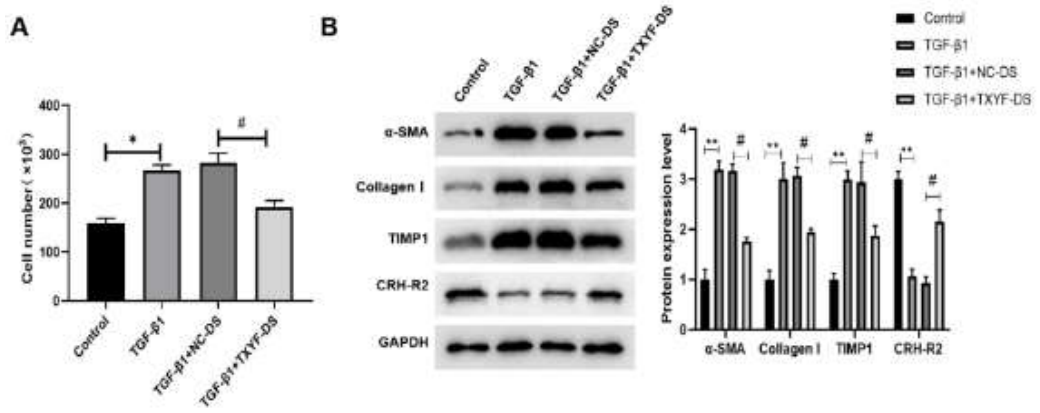

Figure 3: Impacts of TXYF-DS on the cell viability and fibrosis degree. (A) Cell viability determined by Trypan blue staining; (B) The levels of fibrosis marker protein and CRH-R2 examined using Western Blot. $^{\#}P < 0.05$ vs. TGF-β1+NC-DS group; $^{*}P < 0.05$, $^{**}p < 0.01$ vs. control group

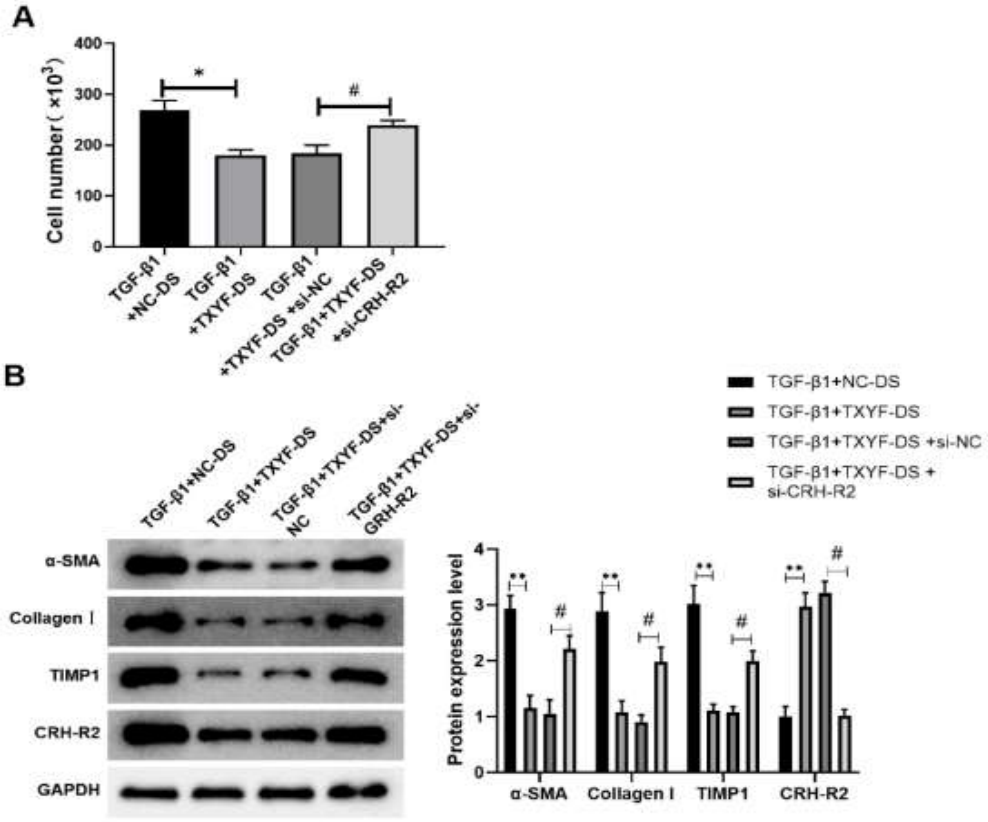

Figure 4: TXYF improves cell fibrosis by regulating CRH-R2. (A) Cell viability determined by Trypan blue staining; (B) The levels of fibrosis marker protein and CRH-R2 determined by Western blot. $^{*}P < 0.05$, $^{**}p < 0.01$ Vs. TGF-β1+NC-DS group; $^{\#}P < 0.05$ vs. TGF-β1+TXYF-DS+si-NC group

TXYF inhibits IL-6/STAT3 signaling through CRH-R2

TXYF has been shown to improve cell fibrosis by regulating CRH-R2 expression. In order to determine the correlation between the expression of CRH-R2 and phosphorylation levels of IL-6/STAT3 pathway-related proteins in the cell model of fibrosis, the levels of related proteins were examined using Western blot. As illustrated in Figure 5, compared to the TGF-β1+NC-DS group, the level of IL-6 and phosphorylation level of STAT3 in the TGF-β1+TXYF-DS group were significantly reduced. This pattern is consistent with the findings observed in the mice model. However, this trend was reversed when CRH-R2 was knocked down. IL-6 and p-STAT3 protein levels were substantially increased, suggesting that TXYF may inhibit the role of IL-6/STAT3 signal pathway by regulating CRH-R2 expression.

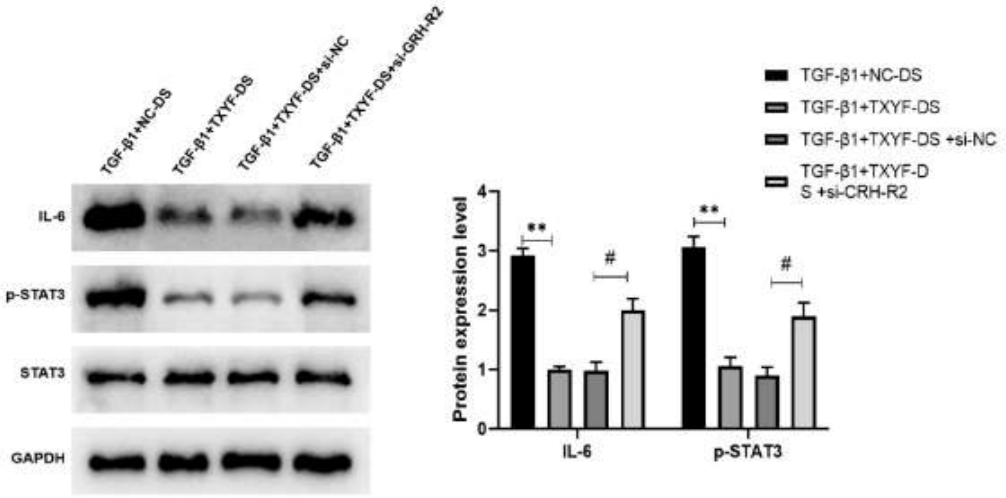

Figure 5: The effect of CRH-R2 knockdown on the activity of IL-6/STAT3 signal pathway (Western blot). ***P* < 0.01 vs. TGF-β1+NC-DS group, $^{\#}P$ < 0.05 vs. TGF-β1+TXYF-DS+si-NC group

DISCUSSION

Intestinal fibrosis is caused by collagen deposition and excessive proliferation of myofibroblasts and has been recognized as a common pathological characteristic of inflammatory intestinal illness. The etiology of bowel fibrosis is usually complex. It is mainly distinguished by the excessive deposition of ECM and proliferation of mesenchymal cells [10]. It is widely assumed that fibrosis is induced by chronic inflammation of colon cells, and delayed repair of the large intestinal organization [18]. In addition, intestinal fibrosis causes frequent lumen stenosis, greatly affecting patients' quality of life [11]. Currently, there is no fully effective strategy for the treatment of fibrosis, thereby necessitating the development of innovative anti-fibrosis interventions.

TGF-β1 plays an important role in fibrosis by inducing ECM sedimentation and proliferation of myofibroblasts. As such, stimulation of CCD-18Co cells with TGF-β1 induce the development of the fibrotic phenotype. Studies have shown that maggot extract (ME) inhibit the TGF-β1/SMAD pathway by up-regulating the Nrf2 expression and improving colitis and the associated intestinal fibrosis induced by the chronic DSS. Metformin inhibits TGF-β1/Smad3 signal transduction pathway, hence improving intestinal fibrosis caused by chronic colitis. In addition, high concentrations of multistrain probiotic lysates inhibited the TGF-β1-induced cell fibrosis [12]. However, TGF-β1 is a versatile and highly regulated cytokine with multiple functions. Inhibiting its expression may be beneficial in inhibiting intestinal fibrosis, but may also result in the occurrence of immune diseases and even tumors [13]. Therefore, the efficacy of anti-TGF-β1 antibodies in combating intestinal fibrosis in clinical settings is constrained due to its sole dependence on the favorable impact of the TGF-β1 intervention. Therefore, identifying effector molecules that are located downstream of TGF-β1 as therapeutic targets may be a more feasible approach.

The corticotropin-releasing hormone (CRH) family mediates functional responses in many organs, including the intestines. For example, it is involved in intestinal mucosal repair and intestinal wall tissue reconstruction. CRH, a brain-intestinal peptide, is primarily secreted by the paraventricular nucleus of the hypothalamus. It is found in abundance in the middle and peripheral gastrointestinal tissues. As an important endocrine hormone in the stress response process, CRH performs various biological functions by binding with different subtypes of corticotrophin-releasing hormone receptors (CRH-R). It participates in the adjustment of neuroendocrine, gastrointestinal automaticity and inflammatory responses [14]. Studies have shown that CRH-R2 not only activates mucosal anti-inflammatory responses and exert anti-inflammatory effects, but also regulates the occurrence and development of epithelial-mesenchymal transition (EMT) by participating in multiple molecular signaling pathways to increase the source of intestinal fibroblasts. In addition, CRH-R2 inhibits the EMT process of colon cancer cells by reducing chronic intestinal inflammation and regulating IL-6/STAT3 pathway, thus reducing distant metastasis of the tumor cells [15].

Relevant animal studies have verified that IL-6 knockout protects mice from fibrosis damage, whereas reducing IL-6 expression inhibits the

peritoneal fibrosis caused by long-term peritoneal dialysis. STAT3 plays a significant role in the fibrosis of multiple organs. For example, in a renal fibrosis model, STAT3 induces EMT in renal tubular epithelial cells, thereby affecting the downstream signaling of TGF-β1. Sustained activation of STAT3 in the intestine has been demonstrated to result in the production of intestinal fibroblasts and acceleration of intestinal fibrosis [16]. These findings suggest that CRH-R2 is more likely to inhibit the EMT process of intestinal epithelial cells through IL-6/STAT3 pathway, thereby improving intestinal fibrosis in inflammatory bowel disease.

Traditional Chinese medicine provides complementary therapies to the traditional remedies for UC. TXYF is composed of white *Rhizoma atractylodes*, *Paeoniae radix* alba, tangerine peel and sand ginseng. The primary herbal medicine used in this recipe is white *Rhizoma atractylodes*, which has a bittersweet and warm nature. It is specifically used to treat spleen deficiency syndrome (SDS), as it nourishes the spleen and eliminates dampness. *Paeoniae radix* alba is a herbal medication that acts as an adjuvant herbal medicine for white *Rhizoma atractylodes*. It is sour and cold and aids in harmonizing the liver and spleen. Its main efficacy is in softening the liver and relieving pain. The tangerine peel is pungent, bitter, and warm, and it is a subsidiary herbal medicine that strengthens the spleen and harmonizes the stomach by regulating the flow of qi and eliminating dampness. The sand ginseng which is mildly cold in nature, serves as a coordinative herbal medicine that guides the action of other herbal medicines in this formula to target the lung and stomach meridians. The combined use of these four herbal medicines relieves diarrhea by invigorating spleen and dissipating dampness, and relieves pain by softening liver and regulating the flow of qi. Several studies have shown that TXYF promotes the restoration of the mucous membrane in mice with colitis by adjusting CRH-R2 and repairing the intestinal mucosa through the Hippo pathway. It also reduces inflammatory responses as well as promoting mucosal healing in rats with UC [17]. These findings indicate that TXYF is a potential treatment for UC. This study focused on investigating the effect and potential mechanism of TXYF on inflammation-associated intestinal fibrosis through a DSS-induced chronic colitis mice model, and a TGF-β1 driven CCD-18Co cell model. It was verified at both animal and cellular level that TXYF ameliorates chronic intestinal fibrosis, which is mainly reflected in the reduction of the protein levels of fibrosis markers alpha-SMA, Collagen I and TIMP1. TXYF significantly reduced the DAI score, and improved histopathological changes and disease symptoms in mice with chronic colitis. At the same time, CRH-R2 showed low expression levels in both the two models of intestinal fibrosis. Additionally, the degree of fibrosis was aggravated after CRH-R2 knockdown, indicating that CRH-R2 regulates fibrosis, while TXYF promotes CRH-R2 expression. Hence, it may be speculated that TXYF has the potential of improving cell fibrosis by promoting CRH-R2 expression. Blocking IL-6/STAT3 signal pathway reduces DSS-induced inflammation in UC rats. In addition, modulating the Th17/Treg equilibrium leads to inhibiting IL-6/STAT3 signaling pathway thereby reducing DSS-induced colitis symptoms in mice [18]. The findings of this study illustrated that TXYF reduces the expression of proteins linked to L-6/STAT3 signaling pathway in fibrotic cell models and phosphorylation level of STAT3, thus hindering the activity of IL-6/STAT3 signal pathway and enhancing fibrosis. However, the knockdown of CRH-R2 significantly attenuated this ameliorative effect, suggesting that TXYF enhances chronic intestinal fibrosis in colitis-affected mice by regulating CRH-R2 to inhibit the activity of IL-6/STAT3 signal pathway.

CONCLUSION

TXYF improve intestinal fibrosis in mice with chronic colitis by up-regulating the expression of CRH-R2 in colonic tissues and CCD-18Co, and inhibiting the activity of IL-6/STAT3 signaling pathway. It enhances chronic intestinal fibrosis in colitis mice by modulating IL-6/STAT3 signaling pathway via the mediation of CRH-R2. Therefore, TXYF is effective in treating chronic intestinal fibrosis in colitis, and its possible mechanism of action is the suppression of IL-6/STAT3 signaling pathway.

DECLARATIONS

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None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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