Original Paper

Xia-Yu-Xue Decoction Inhibits Intestinal Epithelial Cell Apoptosis in CCl₄-Induced Liver Fibrosis

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Key Words

Xia-yu-xue decoction • Cell apoptosis • Tight junction

Abstracts:

Background/Aims: Intestine-derived endotoxin is thought to play a role in the development of liver fibrosis. However, the pathological change in the intestine during liver fibrosis is still poorly understood. Here, we investigated the effects of Xia-yu-xue decoction (XYXD) on intestinal inflammation, apoptosis, and tight junction integrity in the carbon tetrachloride (CCl₄)-induced liver fibrosis. **Methods:** Murine liver fibrosis was developed by CCl₄ treatment three times per week over a 6-week period. The CCl₄-treated mice were divided into two groups: the CCl₄-water group (n=8, CCl₄) and the CCl₄-XYXD group (n=8, CCl₄+XYXD). The CCl₄+XYXD mice were treated with XYXD from the beginning of the first week. The expression of inflammatory cytokines and apoptotic molecules were examined using immunohistochemistry, real-time PCR, and western blot. The intestinal epithelial cell apoptosis was examined by TUNEL staining. The tight junction-related molecules, such as ZO-1, claudin, and occludin in the gut were measured by real-time PCR. **Results:** In CCl₄-treated mice damage of the intestinal epithelia and infiltration of inflammatory cells into the lamina propria and muscular layer were observed. Proinflammatory markers MCP-1, TNF-α, CXCL11, IL-6, and CD68 were significantly increased in the intestinal epithelia in CCl₄-treated mice. The expression of pro-apoptotic molecules including Fas and Bax was increased in the intestinal epithelia in CCl₄-treated mice compared with that in control. The number of TUNEL-positive intestinal epithelial cells was also markedly increased in CCl₄-treated mice. The expression of the tight junction proteins including ZO-1, claudin, and occludin was significantly decreased in CCl₄-treated mice compared with that in control mice. Notably, XYXD treatment ameliorated increased inflammatory markers and apoptosis-related molecules and decreased tight-junction proteins

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in CCl₄-treated mice. **Conclusion:** CCl₄-treatment increased expression of proinflammatory cytokines and pro-apoptotic molecules and disrupted tight junction integrity in the intestine. XYXD treatment ameliorated intestinal inflammation, cell death, and tight junction disintegrity induced by CCl₄ treatment, suggesting that XYXD inhibits CCl₄-mediated liver fibrosis at least in part by ameliorating the intestinal epithelial damage.

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Introduction

Liver fibrosis results from various chronic liver diseases, including chronic hepatitis B and C, autoimmune hepatitis, alcoholic liver disease, and non-alcoholic steatohepatitis. The chronic liver injury causes accumulation of extracellular matrix (ECM), such as collagen in the liver, which develops liver fibrosis and ultimately cirrhosis [1]. The complications of liver fibrosis and cirrhosis include hypersplenism, splenomegaly [2], hepato-renal syndrome [3], portal hypertension [4] and hepatic encephalopathy [5]. Many recent studies suggest that the alterations of intestinal pathophysiology could be associated with liver disease development through their unique anatomical relationship [6]. Previous studies reported that the intestinal microbiome is altered quantitatively as well as qualitatively in patients with cirrhosis compared with healthy individuals [7]. There is evidence that bacterial translocation from the intestinal lumen to mesenteric lymph nodes or other extra intestinal locations play an important role in the pathogenesis of liver fibrosis and its complications, such as gastrointestinal hemorrhage and hepatic encephalopathy [8]. However, the intestinal pathophysiology during liver fibrosis is still poorly understood. Therefore, here we investigated the intestinal pathological changes in carbon tetrachloride (CCl₄)-mediated liver fibrosis mouse model.

Xia-yu-xue decoction (XYXD) was first described in Synopsis of the Golden Chamber in 200 AD [9]. It consists of three medicinal herbs mixed in the ratios in parenthesis as follows: *Radix et Rhizoma Rhei* (10g), *Semen Persicae* (10g), and *Eupolyphaga Seu Steleophaga* (6g). XYXD has been used for the treatment for puerperas suffered from abdominal pain and oligomenorrhea caused by blood stasis. XYXD is often used for the treatment of liver fibrosis and cirrhosis in China. XYXD significantly reduced renal injury associated with bile duct ligation (BDL)-mediated cirrhosis in rats, in which macrophage apoptosis is increased and renal tissue is damaged [10]. However, the mechanism of action of XYXD in liver fibrosis is largely unknown.

In the present study, we investigated the anti-fibrotic effect of XYXD in CCl₄-induced liver fibrosis model. Then, we investigated the intestinal damage in CCl₄-induced liver fibrosis and examined whether XYXD can ameliorate intestinal injury associated with CCl₄-mediated liver fibrosis. We focused on intestinal inflammation, epithelial cell apoptosis, and tight junction integrity. Our study will provide new insight into the understanding of intestinal pathology during liver fibrosis development.

Materials and Methods

Preparation of XYXD

XYXD consisted of crude slices from the following ratios of three medicinal herbs: *Radix et Rhizoma Rhei* (10g), *Semen Persicae* (10g), and *Eupolyphaga Seu Steleophaga* (6g). The medicines were accredited by a certified pharmacologist. The medicinal mixture was extracted twice and infiltrated with 75% ethanol twice, and the resulting ethanol extracts were evaporated and dehydrated under vacuum. The extract powder was weighed and used for the experiments by dissolving in pure water at desired concentrations. XYXD was dissolved in distilled water for *in vivo* and *in vitro* studies.

Major reagents

DAPI, and α -SMA were obtained from Sigma (St Louis, MO, USA). SABC kit for immunohistochemical was from Boster (Wuhan, China). The antibodies used for the immunohistochemical or western blot analysis are goat polyclonal monocyte chemotactic protein 1 (MCP1) (sc-1785) purchased from Santa Cruz Biotechnology (La Jolla, CA, USA), rabbit polyclonal claudin (ab-15098), rabbit polyclonal TNF- α (ab-667). Secondary fluorescence-labeling goat anti-mouse Cy3 (West Grove, PA, USA). Anti-Bax rabbit antibody was from CST (Beverly, MA, USA) and diluted to 1:100 in the studies. Anti-Fas mouse antibody was purchased from BD Pharmingen (San Jose, CA, USA) and prepared in 1:1000 dilution. In situ cell death detection kit TMR red purchased from Roche (Indianapolis, IN, USA). Pierce BCA protein Assay kit was purchased from Thermo Scientific (Rockford, IL, USA), Immobilon-P Transfer membrane from Millipore (Darmstadt, Germany).

Ethics statement

All the study protocols complied with the current ethical considerations of Shanghai University of Traditional Chinese Medicine's Animal Ethic Committee and the procedural and ethical guidelines of the Chinese Animal Protection Act, which is in accordance with the National Research Council criteria. All animal experiments and procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai University of Traditional Chinese Medicine and were performed in accordance with the relevant guidelines and regulations.

Animals

In total, 24 male C57bl mice at (18 $\pm$ 2g) were supplied by the Central Animal Care Facility of Shanghai University of Traditional Chinese Medicine and housed in an air-conditioned room at 25°C with a 12h darkness/light cycle. The mice received humane care with unlimited accesses to chow food and water during the study.

The mice were randomized into two groups: the control (n=8) and CCl₄-treated group (n=16). The CCl₄-treated mice were treated with 10 % CCl₄ (2 mg/kg of body weight i.p.) diluted in olive oil or with olive oil only for triweekly and distilled water (by gavage) daily. The CCl₄-treated mice then divided into CCl₄-water (CCl₄ n=8) and CCl₄-XYXD (CCl₄+XYXD, n=8) from the beginning of first CCl₄-treatment. The XYXD treatment group was exposed to the same level of CCl₄ and administered XYXD at a dose of 0.467g/100 g body weight, which is equivalent to human doses in clinical therapeutics daily for 6 weeks until sacrifice. The number in HE and Sirius red staining was as the same as the animal number in each group.

Liver and gut histopathology

The liver and small intestine were fixed in 10% paraformaldehyde, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin (H&E) and Sirius red.

Quantitative real-time PCR

Total RNA was extracted using Trizol (Takara, Japan), followed by reverse transcription of total RNA to cDNA. Prime Script, RT Reagents Kit (perfect real time), were purchased from Takara (Shanghai, China). cDNA subsequently underwent quantitative real-time polymerase chain reaction (PCR) using the ABI ViiA™ 7Dx real-time PCR system (Life Technologies, NY, USA). PCR primer sequences used were: 18s rRNA forward 5'-AGTCCCTGCCCTTTGTACACA-3'. 18s rRNA reverse 5'-CGATCCGAGGGCCTCACTA-3'. CXCR3 forward 5'-TCTCGTTTCCCAATAATCG-3'. CXCR3 reverse 5'-AGCCAAGCCATGTACCTTGA-3'. TNF- α forward 5'-AGGGTCTGGGCCATAGAAGT-3'. TNF- α reverse 5'-CCACCAGCTCTTCTGTCTAC-3'. IL-1 β forward 5'-AGGTCAAAGGTTTGAAGCA-3'. IL-1 β reverse 5'-TGAAGCAGCTATGGCAACTG-3'. CD68 forward 5'-ACATTGTATTCCACCGCCAT-3'. CD68 reverse 5'-CATCCCCACCTGTCTCTCTC-3'. CXCL11 forward 5'-CATTTTGACGGCTTTCATCC-3'. CXCL11 reverse 5'-AAGGTCACAGCCATAGCCCT-3'. IL6 forward 5'-ACCAGAGGAAATTTTCAATAGGC-3'. IL6 reverse 5'-TGATGCACTTGCAGAAAACAC-3'. Claudin1 forward 5'-ATGCCAATTACCATCAAGGC-3'. Claudin1 reverse 5'-GAGGGACTGTGGATGTCCTG-3'. ZO-1 forward 5'-TGCAATTCCAAATCCAAACC-3'. ZO-1 reverse 5'-AGAGACAAGATGTCCGCCAG-3'. Occludin forward 5'-CATAGTCAGATGGGGGTGGA-3'. Occludin reverse 5'-ATTTATGATGAACAGCCCCC-3'. Gene expression was normalized to 18s rRNA as internal normal.

Western blot

Protein extracts were electrophoresed, blotted, and then incubated with antibodies for Fas, Bax, caspase3, and GAPDH with appropriate secondary horseradish peroxidase (HRP)-conjugated antibodies, and developed.

Immunohistochemistry

The sections were dewaxed in xylene and rehydrated in alcohol. Antigen retrieval was achieved by microwaving in citric saline at 95 °C for 3 min. the sections were treated with 3% hydrogen peroxide for 10 min. The sections were further blocked by 5% BSA and were then incubated at 37 °C with primary antibody against CD68 (Serotec, UK), MCP1 (Santa Cruz, USA) TNF- α (Abcam, UK), Fas (BD, USA), Bax (CST, USA), Claudin 1 (Abcam, UK). The sections were incubated with biotinylated secondary antibody (Boster, Wuhan, China) for 30 min at room temperature. their expressions were visualized by DAB (Boster, Wuhan, China) staining.

Immunofluorescence staining

For gut apoptosis detection, we performed double immunofluorescence staining with terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL). First, we detected TUNEL using a commercially available kit following the manufacturer's instructions. Images were captured using an Olympus BX43 microscope (Olympus, Tokyo, Japan) and processed using DP73 version software.

Statistics

Differences between the two groups were compared using two-tailed unpaired Student's t-test. Differences between multiple groups were compared using one-way ANOVA with a post hoc Dunnett's test using Graphpad prime 5.0. A *P* value of less than 0.05 was considered significant. All experiments were performed at least three times and the representative data were presented.

Results

XYXD inhibited hepatic collagen deposition in liver fibrosis

CCl₄ is well-known to mediate liver damage by producing various reactive metabolites, which significantly damage hepatocytes. Chronic hepatocyte damage caused by CCl₄ treatment eventually induces fibrosis [11]. Mice after the treatment with CCl₄ for 6 weeks displayed marked increases in inflammatory cell infiltration, hepatic steatosis, collagen deposition, and disruption of the lobular liver architecture (Fig. 1A). XYXD improved a number of the parameters related to liver injury and fibrosis. Blood ALT levels were significantly increased following CCl₄ treatment (*P*<0.05). XYXD markedly reduced blood ALT levels in CCl₄-treated mice (*P*<0.05) (Fig 1B). Fibrous collagen deposition was examined by Sirius red staining (Fig. 1C). There was scarcely collagen staining in the normal liver; collagen deposition was barely observed except in the vascular smooth muscle cells. The accumulation of fibrous collagen fiber was evident in CCl₄-treated mice. The bridging fibrosis observed in the CCl₄-treated group (Fig. 1C and 1D), was reduced in the group treated with XYXD (*P*<0.05).

XYXD inhibited hepatic stellate cell activation

CD68, a marker for activated macrophages, was markedly increased in CCl₄-treated mice while XYXD treatment inhibited CD68 expression significantly. The expression of CXCR3, TNF- α , IL-1 β and MCP1 was markedly increased in CCl₄-induced liver fibrosis. The pro-inflammatory cytokines upregulated by CCl₄ treatment were significantly decreased by the XYXD treatment (Fig. 2A, 2B, and 2E-2H).

HSC activation is required for fibrillar collagen production. We examined α -SMA expression as a marker for HSC activation (Fig. 2C). The α -SMA-positive HSCs in incomplete septa and hepatic sinusoids were significantly increased following CCl₄-treatment whereas XYXD treatment reduced α -SMA expression.

The fibrogenic mRNA expression, including TGF- β 1, α -SMA, tissue inhibitor matrix proteinases (TIMP)-1, and Col1(α 1) was also increased after CCl₄ treatment. Expectedly, the

Fig. 1. Effects of Xia-yu-xue decoction (XYXD) on liver histology in CCl₄-induced liver fibrosis in mice. A, HE staining (×100). B, ALT levels in the serum in CCl₄-induced liver fibrosis. C, Sirius red staining (×100). D, quantification of Sirius red staining. CCl₄ (10%, 2mg/kg of body weight) was administered intraperitoneally to CCl₄-treated mice for three times weekly for 6 weeks (CCl₄). The control mice received an equal amount of olive oil for three times weekly for 6 weeks (Control). The XYXD treatment group was exposed to the same amount of CCl₄ and administered XYXD at a dose of 0.467g/100 g body weight (CCl₄+XYXD), which is equivalent to the dose in humans in clinical therapeutics.

Fig. 2. XYXD inhibited HSC activation and the expression of pro-inflammatory and pro-fibrogenic mediators in CCl₄-induced liver fibrosis in mice. A, liver sections of immunohistochemistry for CD68 (n= 6) (×200). B, quantification of CD68 staining (n= 6). C, liver sections of immunohistochemistry for α-SMA (×200). Liver sections were stained with α-SMA (red) and nucleus (DAPI) (n= 6). D, quantification of α-SMA staining (n= 6). E-H, the expression of CXCR3, TNF-α, IL-1β and MCP1 were examined by real-time PCR, I-L, the expression of TGFβ1, α-SMA, TIMP1, and Col 1(α1) were examined by real-time PCR. As an internal control, 18S rRNA was used. The data are reported as means ± SD.

Fig. 3. Effects of XYXD on gut macrophages and pro-inflammatory cytokines in mice with liver fibrosis. A, gut HE staining ($\times 200$) B, gut sections of immunohistochemistry for MCP1 ($n = 6$) ($\times 200$). C, quantification of MCP1 staining. D, gut sections of immunohistochemistry for TNF- α ($n = 6$) ($\times 200$). E, quantification of TNF- α staining. F-H, the expression of CD68 (F), CXCL11 (G), and IL6 (H) was measured by real-time PCR ($n = 6$). The data are reported as means $\pm$ SD.

fibrogenic gene expression was clearly reduced after XYXD administration. Taken together, these results demonstrated that XYXD treatment suppressed CCl₄-induced activation of Kupffer cells and HSCs and production of collagen in the liver.

The intestinal epithelia were damaged in CCl₄-treated mice

The intestine-derived endotoxin is known to be involved in the development of liver diseases, such as liver fibrosis [12] and alcoholic liver disease [13]. It has been suggested that gut microbe is strongly associated with induction of intestinal damage while extensive analysis of pathological changes in the intestine during the development of liver injury or fibrosis has not been studied well. Thus, we wanted to examine the pathological changes in the intestine during CCl₄-induced liver fibrosis. HE staining shows few inflammatory cell infiltration in control mice (Fig. 3A). In the intestine of CCl₄-treated mice, a significant inflammatory cell infiltration was observed whereas XYXD treatment inhibited inflammatory cell infiltration.

The pro-inflammatory cytokines were increased in the small intestine in CCl₄-treated mice

Next, we wanted to know whether inflammatory cytokines are increased in the gut. In normal mice, MCP1 is weakly expressed and located in the villi of the small intestine and the epithelial cells almost did not express MCP1 as shown by immunohistochemistry (Fig. 3B). In contrast, MCP1 expression was markedly upregulated not only in villi but also in epithelial

Fig. 4. Effects of XYXD on gut pro-apoptotic molecules in mice with liver fibrosis. A, gut sections of immunohistochemistry for Bax ($n=6$) ($\times 200$). B, quantification of Bax staining. C, gut sections of immunohistochemistry for Fas ($n=6$) ($\times 200$). D, quantification of Fas staining. E, western blot analysis of Fas, Bax, and cleaved-caspase 3 ($n=6$). F-H, quantification of Fas, Bax, and cleaved-caspase3.

cells in the mice after CCl_4 treatment. Notably, the XYXD treatment dramatically inhibited MCP1 expression (Fig. 3B and 3C). Similar to MCP1 expression, $\text{TNF-}\alpha$ expression was weak in the villi of the small intestine in normal mice whereas MCP-1 levels were increased in the gut of the mice following CCl_4 treatment (Fig. 3D and 3E). The XYXD treatment significantly reduced $\text{TNF-}\alpha$ expression in the intestine.

We also examined the mRNA expression of CD68, CXCL11, and IL6 in the small intestine in CCl_4 -treated mice (3F-3H). Consistent with the expression of pro-inflammatory cytokines examined by immunohistochemistry, the mRNA levels of pro-inflammatory markers (CD68, CXCL11, and IL-6) were significantly increased following CCl_4 -treatment, and they were remarkably reduced by the XYXD treatment.

The apoptosis was increased in the small intestine in CCl_4 -treated mice

Cell death plays a key role in the pathology associated with inflammatory diseases [14]. Next, we wanted to examine whether apoptosis is associated with intestinal damage following the CCl_4 treatment. We first examined a proapoptotic BAX protein expression by immunohistochemistry, Bax was barely expressed in the small intestine from control mice. After CCl_4 treatment, BAX expression was increased and mainly located in epithelial cells of the villi and the glands of the small intestine (Fig. 4 A and 4B). Interestingly, the localization of the Fas expression was distinct from that of the Bax expression. In control mice, Fas expression was weak and expressed in the submucosa and lamina propria of the small intestine. Upon CCl_4 treatment, the Fas expression was strongly upregulated and its expression was mainly located in epithelial cells and lamina propria (Fig. 4C and 4D). The Bax and Fas expression was significantly reduced in the XYXD-treated mice (Fig. 4A-D).

We also examined the expression of Fas, Bax, and cleaved-caspase-3 through Western blots (Fig. 4E-H). The expression of these apoptosis-related molecules in the small intestine

Fig. 5. Effects of XYXD on intestinal epithelial cell apoptosis in mice with liver fibrosis. A, TUNEL were stained with TMR red. The nucleus was stained by DAPI (n= 6) ($\times 200$). B, quantification of TUNEL staining.

Fig. 6. Effects of XYXD on the gut tight junction in mice with liver fibrosis. A, gut sections of immunohistochemistry for claudin (n= 6) ($\times 200$). B, quantification of claudin staining. C-E, the mRNA expression of claudin (C), ZO-1 (D), and occludin (E) were examined by real-time PCR (n= 6).

was increased after CCl₄-treatment while the expression of these molecules was significantly reduced by the XYXD treatment in the mice treated with CCl₄.

Epithelial cells of the villi of the small intestine underwent apoptosis in CCl₄-treated mice

The increased expression of pro-apoptotic molecules is associated with cellular apoptosis. Here, we evaluated epithelial cell apoptosis in the small intestine using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay (TUNEL). There

were few apoptotic cells in the small intestine in normal mice (Fig. 5). In contrast, apoptotic cells were significantly increased in the small intestine in CCl₄-treated mice. The apoptotic cells were mainly observed in the epithelial cells of the villi. The XYXD treatment reduced cellular apoptosis remarkably (Fig. 5A and 5B).

The tight junction integrity of intestinal epithelial cells was destroyed in CCl₄-treated mice

The increased intestinal epithelial cell apoptosis could result in the destruction of the normal structure of intestine [15]. We examined the expression of tight junction molecules in the small intestine in CCl₄-treated mice. The expression of claudin was very strong in epithelial cells in the villi and gland of the gut in normal mice. However, the claudin expression was significantly decreased in CCl₄-treated mice. The XYXD treatment reversed the claudin expression that was reduced by CCl₄-treatment (Fig. 6A and 6B). The real-time PCR analysis also confirmed the results from immunohistochemistry. The expression of other tight junction molecules ZO-1 and occludin displayed similar trend with the claudin expression in CCl₄-treated mice (Fig. 6C-E).

Discussion

It has been reported that the gut-liver axis plays an important role in the development of liver fibrosis and cirrhosis. However, the pathological changes in the intestine during the development of liver fibrosis was not described precisely. The present study aims to demonstrate pro-inflammatory cytokine production, epithelial cell, and tight junction damage in the intestine in mice with liver fibrosis. We also found that the XYXD administration attenuated intestinal epithelial inflammation and damage at least in part by ameliorating the intestinal epithelial tight junction integrity.

Hepatic fibrosis is characterized by progressive inflammation and excessive deposition of extracellular matrix (ECM) [1, 11]. Hepatic stellate cells (HSCs) the main collagen-producing cells in the damaged liver. Activation of HSCs is the key process for the development of liver fibrosis after the chronic liver injury associated with any etiology [7, 16]. Although it is a well-accepted concept that HSCs are the main target cells for the treatment of hepatic fibrosis, effective therapies for liver fibrosis have not been established yet. It would take very long duration for the establishment of liver fibrosis and its mechanisms are very complex. In addition, the pathology of extrahepatic organs may also be involved in the development of liver fibrosis. For example, it has been reported that the kidney injury is often observed in patients with liver fibrosis induced by hepatitis C [17]. We have also reported that macrophage infiltration and apoptosis in the kidney of fibrotic and cirrhotic rats [18, 19]. The pathological changes in extrahepatic organs, such as spleen, lung [20], and heart [21] associated with liver fibrosis have also been reported. However, the pathological changes in the intestine during the development of liver fibrosis were poorly understood.

Increased evidence show that the gut-liver axis participates in the development of liver injury [22]. The disruption of the intestinal barrier can enhance the exposure of intestine-derived microorganisms and/or microbial products to the liver. This may lead to either detrimental or beneficial effect, which is organ and context-dependent [23]. Primary sclerosing cholangitis (PSC) is a devastating liver disease strongly associated with inflammatory bowel disease (IBD). It has been suggested a crosstalk of the immune reactions between the gut and the liver [24]. It has also been reported that abnormal composition of gut microbiota was observed in patients with cystic fibrosis [25]. Alcoholic liver disease (ALD) is also associated with gut disturbances, both functionally and structurally. It is well-accepted that the gut-liver axis plays an important role in the prevention of systemic inflammation as well as liver health [26-28]. The data reported previously suggest that the pathological changes in the gut strongly participate in the progression of liver injury. However, the precise pathological findings in the gut during the development of liver fibrosis have not been described well previously.

The alterations of gut microbiota are associated with the development of an inflammatory environment, fibrosis progression and bacterial translocation in carbon tetrachloride-treated mice [8]. It has been reported that the oral administration of *S. boulardii* improved the liver function in CCl₄-treated rats, and the preventive treatment of this probiotic yeast suppressed the progression of liver fibrosis [7]. Liver transplant has also been reported to improve the composition of the gut microbiome and cognitive impairment in cirrhotic patients [12]. Increased translocation of bacteria and bacterial products from the intestine may impair liver homeostasis and enhance liver inflammation through activation of the innate immune system [11, 29]. Specifically, activation of TLR4 stimulates macrophages and HSC activation. Although researchers did a plenty of works in how gut endotoxin affects liver injury, there were few reports showing the pathological changes in the gut in the development of liver fibrosis.

Here, we demonstrate that proinflammatory cytokine production and inflammatory cell infiltration was increased in the intestine in CCl₄-induced liver fibrosis, which is in agreement with other reports [29, 30]. However, there are still a big gap between inflammation and gut barrier dysfunctions. Our study demonstrated that inflammatory cytokines, such as TNF-α, MCP1, and IL6, were significantly increased in CCl₄-treated mice when we compared with control mice. It has been reported that TNF-α contributes to the gut epithelial cell apoptosis [31], dysregulation of the functions tight junctions, and the loss of intestinal epithelial barrier [32]. Then, bacteria and toxic substances destroy the intestinal lumen physically and functionally and endotoxin will be translocated to the liver and HSC activation will be induced.

Conclusion

The present study demonstrated that Xia-yu-xue decoction can improve the histological changes and reduce ECM accumulation in CCl₄-induced liver fibrosis. The relevant mechanism involves inhibiting gut epithelial cell apoptosis and tight junction destruction. These results suggest that liver fibrosis is not only restricted to the liver but also involved in the gut. Gut epithelial cell apoptosis and following the tight junction disruption were observed in CCl₄-induced liver fibrosis. The Xia-yu-xue decoction administration can attenuate CCl₄-induced liver fibrosis at least in part by regulating epithelial inflammation, cell apoptosis, and tight junction integrity.

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Disclosure Statement

The authors declare no Disclosure Statement.

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