Research Article

Jianpi Qingchang Decoction Ameliorates Chronic Colitis in Piroxicam-Induced IL-10 Knockout Mice by Inhibiting Endoplasmic Reticulum Stress

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Background. Excessive endoplasmic reticulum (ER) stress in intestinal epithelial cells (IEC) may lead to impaired intestinal mucosal barrier function and then participate in the pathogenesis of ulcerative colitis (UC). Jianpi Qingchang decoction (JPQCD) has been shown to have protective effects on UC. However, further studies are needed to determine whether JPQCD regulates PERK/eIF2α/ATF4/CHOP pathways to play a role in treating UC.

Methods. IL-10−/− mice were randomly assigned into five groups: control, model, low-dose JPQCD (JPQCDL), middle-dose JPQCD (JPQCDM), and high-dose JPQCD (JPQCDH). All groups except for the control group were given model feed containing 200 ppm piroxicam for 10 d to induce colitis. As a comparison, we used wild-type mice that were the progeny of IL-10+/− matings, bred in the same facility. The control group and wild-type mice were fed with common feed. At the same time, mice in each group were given corresponding drugs by gavage for 14 d. The disease activity index of mice in each group was evaluated daily. Colon tissues of mice were collected, colon length was measured, and pathological changes and ultrastructure of colon epithelial cells were observed. The effects of JPQCD on the PERK/eIF2α/ATF4/CHOP pathways were evaluated by western blotting and reverse transcription-polymerase chain reaction (RT-PCR). The expression of CHOP in colon tissue was detected by tissue immunofluorescence assay. The expression of NF-κB, p-NF-κB p65 protein was analyzed by western blotting; the level of IL-17 in colon tissue was detected by enzyme-linked immunosorbent assay (ELISA) and verified by examining NF-κB and IL-17 mRNA levels by RT-PCR.

Results. Compared with the control group, the model group showed significant colitis symptoms and severe colonic tissue damage. The results showed that JPQCD significantly reduced body weight loss, ameliorated disease activity index, and restored colon length in IL-10−/− mice with piroxicam-induced colitis. Western blotting and RT-PCR showed that the PERK/eIF2α/ATF4/CHOP pathway was activated in colon tissue of model mice, suggesting that the pathway is involved in the pathogenesis of ulcerative colitis (UC) and could become a potential therapeutic target. The JPQCD treatment inhibited the activation of the PERK/eIF2α/ATF4/CHOP pathway, alleviated the ER stress, and played a role in preventing and treating UC. In addition, JPQCD can also downregulate the protein of NF-κB, p-NF-κB p65, downregulated the mRNA expression of NF-κB, and reduce the content of IL-17 and its mRNA expression in colon tissues.

Conclusion. JPQCD may play a protective role in UC by regulating the PERK/eIF2α/ATF4/CHOP signaling pathway and relieving endoplasmic reticulum stress.
1. Introduction

Ulcerative colitis (UC) is a chronic nonspecific intestinal inflammatory disease characterized by continuous and diffuse inflammatory changes in the colorectal mucosa. Its lesions are mainly confined to the large intestinal mucosa and submucosa. Clinical manifestations are diarrhea, mucous pus and blood in the stools, and abdominal pain. The severity of the disease varies, and most cases show a chronic course of recurrent attacks [1]. The incidence and prevalence of UC are increasing worldwide. The etiology of UC is not clear, and it is generally considered to be related to factors such as heredity, environment, intestinal microecology, and immune imbalance [2]. So far, aminosalicylic acid preparations, corticosteroids, and immunosuppressants are the main drugs for the treatment of UC. However, these drugs are accompanied by a variety of potential adverse effects [3]. As an alternative and complementary medicine for the treatment of inflammatory bowel disease (IBD), traditional Chinese medicine (TCM) has unique advantages for the prevention and treatment of IBD due to its efficacy and safety [4, 5].

It has been shown that various causes contribute to intestinal epithelial cell (IEC) endoplasmic reticulum (ER) stress and activate the unfolded protein response (UPR), which collectively participate in the development of IBD [6, 7]. UPR is controlled by three major sensors: pancreatic ER eIF2α kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) [8]. In the absence of ER stress, glucose-regulated protein 78 (GRP78) binds to the luminal domains of the ER stress sensors and is maintained in an inactive state [9]. Upon ER stress, GRP78 dissociates from three proteins, thus activating PERK, IRE1, or ATF6 and starting UPR, thus promoting the correct folding of proteins and inhibiting the synthesis of proteins [10]. Among the three pathways of ER stress, the PERK pathway mainly recognizes and interacts with a variety of unfolded proteins to regulate protein synthesis [11].

Jianpi Qingchang decoction (JPQCD) is composed of Coptis chinensis Franch. (Huang Lian) 3 g, Astragalus mongholicus Bunge (Huang Qi) 30 g, Codonopsis pilosula (Franch.) Nannf. (Dang Shen) 15 g, Portulaca oleracea L. (Ma Chi Xian) 30 g, Sanguisorba officinalis L. (Sheng Di Yu) 15 g, Panax notoginseng (Burkii) F. H. Chen (San Qi) 6 g, Bletilla striata (Thunb.) Rchb.f. (Bai Ji) 3 g, Aucklandia costus Falc. (Mu Xiang) 6 g, and Glycyrrhiza uralensis Fisch. ex DC. (Gan Cao) 6 g. Previous studies have shown that JPQCD can significantly alleviate the clinical symptoms of patients with mild-to-moderate active UC and thus improve their quality of life [12]. Previous experimental studies have shown that JPQCD can significantly improve the symptoms of dextran sodium sulfate- (DSS-) induced colitis, and the mechanism may be related to the inhibition of nuclear factor-κB activation, downregulation of inflammatory mediators such as interleukin- (IL-)1β, IL-8, tumor necrosis factor-α, and improvement of colonic epithelial barrier function in mice [13, 14]. Moreover, we found that JPQCD can also regulate DSS-induced abnormal intestinal motility in UC mice by inhibiting intestinal inflammatory cascading and reducing autophagy of Cajal stromal cells [15]. However, it is not clear whether JPQCD can regulate the PERK/eIF2α/ATF4/CHOP pathway. In this study, we used piroxicam to induce IL-10−/− mice to produce a chronic colitis model and explored whether JPQCD could regulate the PERK/eIF2α/ATF4/CHOP pathway, thus improving ER stress and preventing UC.

2. Materials and Methods

2.1. Animals and Experimental Design. IL-10−/− mice on a C57BL/6 strain background were obtained from Shanghai Model Organisms (license No. SCXK (Shanghai) 2017-0010). Mice were bred and mated in SPF Animal Experimental Center of Shanghai University of Traditional Chinese Medicine (license No. SCXK (Shanghai) 2020-0009), under standard conditions (room temperature, 24 ± 2°C; humidity 50%–60%; 12 h light/dark cycle). Shanghai Model Organisms provided genetic testing services. This study was reviewed and approved by the Experimental Animal Ethics Committee of Shanghai University of Chinese Medicine (PZSHUTCM190912020).

Using SPSS version 25.0 to generate random numbers, IL-10−/− mice were randomly assigned to five groups: control, model, low-dose JPQCD (JPQCD L), middle-dose JPQCD (JPQCD M), and high-dose JPQCD (JPQCD H) (n = 8, 4 male and 4 female). Chow containing piroxicam (Sigma–Aldrich, St. Louis, MO, USA) was fed to IL-10−/− mice for 10 d at a dose of 200 ppm to induce colitis [16]. For comparison, we used wild-type mice that were the progeny of IL-10−/− matings, bred in the same facility. Control and wild-type mice were fed a common diet. Mice in the treatment group were given JPQCD (10, 15, or 22.5 g/kg/d) by gavage for 2 wk, and mice in the model group, control group, and wild-type group were given normal saline by gavage. The animal experiment was conducted in accordance with the Laboratory Animal Regulations of the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine and was approved by the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine. During the experiment, the body weight, stool characteristics, and fecal occult blood of mice in each group were recorded every day. The disease activity index (DAI) refers to Sánchez’s method and is described in Table 1, which is composed of the mean value of the sum of the three indexes [17].

2.2. Preparation of JPQCD. The nine medicinal herbs contained in JPQCD (listed above) were purchased from Longhua Hospital Shanghai University of Traditional Chinese Medicine (Shanghai, China). After soaking the above medicines, they were decocted twice, filtered, and concentrated to a volume of 600 mL. After being concentrated in a rotary evaporator, the concentrated JPQCD was placed in a freeze-drying machine to prepare freeze-dried powder, which was sealed and stored at −20°C.
The extracted RNA was reverse transcribed to cDNA using a reverse transcription kit (Takara, Kyoto, Japan) and RT-PCR was conducted based on the Eppendorf PCR system. The mRNA expression of the following genes was assessed using qPCR: β-actin, PERK, eIF2α, GRP78, AT4, CHOP, NF-κB, IL-17. The relative expression of target genes was calculated using the ∆∆Ct method. Primer sequences are presented in Table 2.

2.7. Western Blotting. Frozen colon tissue samples were homogenized in lysis buffer to obtain total protein. Protein concentration was determined in supernatants using the BCA Protein concentration determination kit (Beyotime, Shanghai, China). Equal amounts of protein were separated by SDS-PAGE and transferred to the polyvinylidene difluoride membrane. The membranes were blocked in 5% nonfat milk for 1 h, and membranes were incubated at 4°C overnight with primary antibodies to p-PERK, eIF2α, p-eIF2α, CHOP, NF-κB, β-actin (from Cell Signaling, Danvers, MA, USA), GRP78 (from Proteintech, Wuhan, Hubei, China), AT4 (from Santa Cruz, Dallas, TX, USA), and p-NF-κB p65 (from ABclonal, Wuhan, Hubei, China). The membranes were incubated with secondary antibodies, anti-rabbit IgG or anti-mouse IgG (from Cell Signaling, Danvers, MA, USA) for 1 h at room temperature. Protein bands were quantified using ImageJ and the grey value of the targets was normalized by β-actin.

2.8. Immunofluorescence. To investigate localization of CHOP, paraffin sections of the colon were dewaxed to water, antigen repaired, blocked, and then incubated with CHOP (1:400) overnight. The secondary antibody was incubated at room temperature for 1 h and then stained with DAPI for 2 min.

2.9. Enzyme-Linked Immunosorbent Assay. The colon tissue of 50 mg mice was weighed and added 1 mL of precooled PBS. After homogenization, the supernatant was centrifuged at 5000xg for 5 min. Experiments were done following the instructions of the Mouse IL-17 ELISA kit (from Elabscience, Wuhan, Hubei, China).

2.10. Statistical Analysis. All data are presented as mean ± SEM. Statistical differences between different groups were measured through one-way ANOVA. Results were considered statistically significant at \( P < 0.05 \). Data analyses were conducted using SPSS version 25.0.

3. Results

3.1. Phytochemicals Identification of JPQCD. Forty-four phytochemicals in JPQCD were identified by UPLC-Q-TOF/MS-based on multistage mass spectrum information of samples, high-resolution mass spectrum database of natural products, and relevant literature (Table 3). Among them, 9 compounds were from Glycyrrhiza uralensis Fisch. ex DC., 8 compounds from Sanguisorba officinalis L., and 8 compounds from Sclerocarya birrea Hochst. ex A. Rich.
compounds from *Panax notoginseng* (Burkill) F. H. Chen. Five compounds are derived from *Bletilla striata* (Thunb.) Rchb.f. Then, there were 4, 3, 3, 2, and 1 compounds derived from *Coptis chinensis* Franch., *Portulaca oleracea* L., *Astragalus mongholicus* Bunge, *Aucklandia costus* Falc., and *Codonopsis pilosula* (Franch.) Nannf. The UPLC-Q-TOF/MS chromatographic profile is shown in Figure 1.

3.2. JPQCD Alleviated the Symptoms of Experimental Chronic Colitis in IL-10<sup>−/−</sup> Mice. We established a mouse model of chronic colitis by adding 200 ppm piroxicam feed to IL-10<sup>−/−</sup> mice for 10 d to evaluate the therapeutic effect of JPQCD (Figure 2(a)). IL-10<sup>−/−</sup> mice exposed to piroxicam showed significant weight loss, diarrhea, and blood in the stools. DAI of mice in the model group was higher than that in the wild-type group and control group, and JPQCD treatment significantly increased body weight and reduced DAI (Figures 2(b) and 2(c)). Colonic shortening indirectly reflected the pathological process of colitis, and JPQCD significantly increased the colon length in the model group (Figure 2(d)).

3.3. JPQCD Inhibited Intestinal Inflammatory Infiltration of Experimental Chronic Colitis in IL-10<sup>−/−</sup> Mice. H&E staining showed that the colon crypts of wild-type mice were normal, with abundant goblet cells, few lamina propria monocytes, and no sign of mucosal thickening or ulceration. In the model group, there was a lot of inflammatory cell infiltration, mucosal thickening, goblet cell reduction, obvious ulceration, and irregular glandular arrangement in the colonic tissues. The JPQCD-treated mice showed intact colonic structures, no obvious ulcers, and less inflammatory cell infiltration (Figures 3(a)–3(f)).

3.4. JPQCD Reduced ER Stress in IECs of Mice with Piroxicam-Induced Colitis. The colonic epithelial cells were observed under transmission electron microscopy. In the wild-type and control groups, the ER showed a membranous network structure, clear and in clumps; the omental cavity was not expanded, and a large number of ribosomes were attached to it; and the cells had many mitochondria with a light color. In the model group, the rough ER (RER) was increased, and the ER cavity was significantly expanded. There were many ER cavities of different sizes and shapes, which were vacuolated and partially fused into clusters. In the JPQCD group, the RER was reduced compared with that in the model group and slightly dilated with less quantity, and the morphology tended to be reticular. In conclusion, JPQCD improved ER stress in IECs of mice with colitis (Figures 4(a)–4(f)).

3.5. JPQCD Regulated Gene Expression Related to PERK/eIF2α/ATF4/CHOP Signaling Pathway. To further investigate the effect of JPQCD on piroxicam-induced colitis, we performed an RT-PCR analysis of several signaling molecules. Compared with the control group, the mRNA levels of PERK, eIF2α, GRP78, ATF4, and CHOP in the model group were higher. The mRNA expression of PERK, eIF2α, GRP78, ATF4, and CHOP in the colon of the JPQCD group was significantly lower than that in the model group. These results suggested that JPQCD-induced improvement in experimental colitis in mice was associated with the PERK/eIF2α/ATF4/CHOP signaling pathway (Figures 5(a)–5(f)).

3.6. JPQCD Regulated Expression of Proteins Related to the PERK/eIF2α/ATF4/CHOP Signaling Pathway. To further investigate the anti-inflammatory mechanism of JPQCD, we detected the protein expression of key signaling molecules in the PERK/eIF2α/ATF4/CHOP pathway. Western blotting showed that PERK, eIF2α, GRP78, ATF4, and CHOP proteins were highly expressed in the model group, while JPQCD treatment significantly reduced the expression of these proteins. The results showed that the improvement of experimental colitis symptoms in mice induced by JPQCD was associated with inhibition of the PERK/eIF2α/ATF4/CHOP signaling pathway (Figures 6(a)–6(f)). Immunofluorescence staining further confirmed that JPQCD could depress CHOP expression in piroxicam-induced colitis mice (Figure 6(g)).

3.7. JPQCD Inhibited the Activation of NF-κB and IL-17. NF-κB is considered to be the key molecular pathway of UC. We detected the effects of JPQCD on the expression of NF-κB by Western blot. The expression of NF-κB protein increased significantly in the colon of piroxicam-induced colitis, which was inhibited by JPQCD treatment (Figure 7(a)). The protein level of p-NF-κB p65 reflected its transcription in the nucleus, and JPQCD significantly inhibited the phosphorylation level of p65 (Figure 7(b)). Since IL-17 is a cytokine with a strong proinflammatory activity involved in the pathogenesis of a variety of chronic inflammatory diseases, we used ELISA to detect the level of IL-17 in colonic tissues. A significant elevation of IL-17 content was observed in model mice compared with the control mice. The elevated IL-17 level was significantly decreased in colitis mice treated with JPQCD (Figure 7(c)). To further verify the inhibitory effects of JPQCD on NF-κB and IL-17, we determined the mRNA levels of NF-κB and IL-17 in the colon by RT-PCR. The results showed that the mRNA expressions of NF-κB and IL-17 in the JPQCD group were significantly lower than those in the model group.
Table 3: Identification results of main components of JPQCD.

| NO | Retention time (min) | Adducts | Measured M/Z | Expected M/Z | ppm | Formula | Molecular weight | Phytochemical name | MS/MS spectra |
|----|----------------------|---------|--------------|--------------|-----|---------|------------------|-------------------|---------------|
| 1  | 1.81                 | [M-H]^- | 169.0158     | 169.0142     | 9.2 | C_7H_6O_5 | 170.02           | Gallic acid       | 169.0168; 125.0250; 81.0368 |
| 2  | 5.29                 | [M-H]^- | 345.0832     | 345.0827     | 1.4 | C_{13}H_{18}O_{10} | 346.09            | Methyl 6-O-galloyl-β-D-glucoside | 345.0814; 313.0536; 124.0170 |
| 3  | 9.15                 | [M-H]^- | 289.0737     | 289.0718     | 6.7 | C_{13}H_{14}O_{6} | 290.08            | Catechin          | 289.0721; 271.0594; 245.0833; 203.0725; 151.0401; 277.0032; 197.0455; 182.0225; 166.9997; 123.0086; 289.0737; 245.0844; 203.0715; 151.0419; 511.2700; 465.2368; 333.1949; 311.0997; 251.0743; 417.1190; 255.0670; 135.0101; 119.0510 |
| 4  | 11.26                | [M-H]^- | 277.0034     | 277.0024     | 3.7 | C_{9}H_{10}O_{8}S | 278.01           | Gallic acid ethyl sulfate | 277.0032; 197.0455; 182.0225; 166.9997; 123.0086; 289.0737; 245.0844; 203.0715; 151.0419; 511.2700; 465.2368; 333.1949; 311.0997; 251.0743; 417.1190; 255.0670; 135.0101; 119.0510 |
| 5  | 12.54                | [M-H]^- | 289.0735     | 289.0718     | 6.0 | C_{13}H_{14}O_{6} | 290.08            | Epicatechin        | 289.0721; 271.0594; 245.0833; 203.0725; 151.0401; 277.0032; 197.0455; 182.0225; 166.9997; 123.0086; 289.0737; 245.0844; 203.0715; 151.0419; 511.2700; 465.2368; 333.1949; 311.0997; 251.0743; 417.1190; 255.0670; 135.0101; 119.0510 |
| 6  | 18.78                | [M+FA-H]^- | 511.2434 | 511.2396     | 7.4 | C_{21}H_{38}O_{11} | 466.24           | Rhodioloside E    | 511.2700; 465.2368; 333.1949; 311.0997; 251.0743; 417.1190; 255.0670; 135.0101; 119.0510 |
| 7  | 20.40                | [M-H]^- | 417.1220     | 417.1191     | 6.9 | C_{22}H_{22}O_{8} | 418.13           | Liquiritin         | 417.1190; 285.0746; 270.0507; 619.2267; 439.1630; 171.0665; 153.0568; 549.1600; 255.0650; 135.0084 |
| 8  | 20.41                | [M+H]^+  | 447.1256     | 447.1286     | -6.7 | C_{22}H_{22}O_{10} | 446.12          | Calycosin-7-O-β-D-glucoside | 445.0814; 313.0536; 124.0170 |
| 9  | 21.52                | [M-H]^- | 619.2303     | 619.2244     | 9.6 | C_{23}H_{40}O_{16} | 620.23          | Dactylorhin E      | 619.2267; 439.1630; 171.0665; 153.0568; 549.1600; 255.0650; 135.0084 |
| 10 | 21.57                | [M-H]^- | 549.1631     | 549.1614     | 3.2 | C_{23}H_{30}O_{13} | 550.17          | Liquiritin apioside | 549.1600; 255.0650; 135.0084 |
| 11 | 22.16                | [M+H]^+  | 504.1466     | 504.15       | -6.8 | C_{23}H_{23}NO_{11} | 503.14          | Oleracein A        | 504.15; 147.0431; 85.0268 |
| 12 | 24.67                | [M+H]^+  | 534.159      | 534.1606     | -3  | C_{23}H_{27}NO_{12} | 533.15          | Oleracein B        | 533.15; 147.0431; 85.0268 |
| 13 | 27.62                | [M-H]^- | 457.1737     | 457.1715     | 4.7 | C_{21}H_{30}O_{11} | 458.18          | Gymnoside II       | 457.1737; 285.0976; 153.0576; 127.0770; 320.0907; 292.0961; 277.0757; 249.0733; 887.3237; 619.2244; 439.1595; 179.0563 |
| 14 | 28.37                | [M-H]^- | 457.1753     | 457.1715     | 8.2 | C_{21}H_{30}O_{11} | 458.18          | Gymnoside I        | 457.1737; 285.0976; 153.0576; 127.0770; 320.0907; 292.0961; 277.0757; 249.0733; 887.3237; 619.2244; 439.1595; 179.0563 |
| 15 | 28.55                | M^+     | 320.0897     | 320.0917     | -6.4 | C_{18}H_{14}NO_{4} | 320.09          | Coptisine          | 320.0917; 292.0961; 277.0757; 249.0733; 887.3237; 619.2244; 439.1595; 179.0563 |
| 16 | 28.76                | [M+FA-H]^- | 933.3300 | 933.3245     | 5.9 | C_{40}H_{58}O_{22} | 888.33          | Dactylorhin A      | 888.33; 179.0563 |
Table 3: Continued.

| NO | Retention time (min) | Adducts          | Measured M/Z   | Expected M/Z | ppm  | Formula       | Molecular weight | Phytochemical name                  | MS/MS spectra |
|----|----------------------|-------------------|----------------|--------------|------|---------------|------------------|-------------------------------------|---------------|
| 17 | 29.04                | M⁺               | 338.1364       | 338.1387     | -6.8 | C₂₀H₂₀NO₄    | 338.14           | Jatrorrhizine                      | 338.1386; 322.1051; 208.0897; 294.1110; 279.0878; 305.1232; 215.1090; 185.0984; 159.0830; 143.0720; 267.0671; |
| 18 | 29.22                | [M + FA-H]⁻       | 441.1797       | 441.1766     | 7.0  | C₂₀H₂₈O₈     | 396.18           | Lobetyolin                        | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 19 | 30.81                | [M + FA-H]⁻       | 475.1273       | 475.1246     | 5.7  | C₂₂H₂₂O₉     | 550.17           | Ononin                             | 252.0399; 223.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 20 | 30.96                | [M-H]⁻             | 549.1657       | 549.1614     | 7.9  | C₁₈H₃₀O₁₃   | 314.1417         | Isoliquiritin apioside             | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 21 | 31.72                | [M + FA-H]⁻       | 977.5402       | 977.5327     | 7.7  | C₄₇H₈₀O₁₈   | 932.53           | Notoginsenoside R1                 | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 22 | 32.16                | [M + H]⁺           | 314.1388       | 314.1387     | 0.4  | C₁₈H₁₉NO₄   | 313.13           | N-trans-Feruloyltyramine           | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 23 | 32.34                | M⁺                 | 352.1528       | 352.1543     | -4.4 | C₂₀H₂₂NO₄   | 352.15           | Palmatine                          | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 24 | 32.44                | M⁺                 | 336.1216       | 336.1230     | -4.3 | C₂₀H₁₈NO₄   | 336.13           | Berberine                          | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 25 | 32.69                | [M + FA-H]⁻       | 771.2747       | 771.2717     | 3.9  | C₃₄H₄₆O₁₇   | 726.27           | Militarine                         | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 26 | 32.83                | [M + FA-H]⁻       | 845.4934       | 845.4904     | 3.5  | C₄₃H₇₂O₁₄   | 800.49           | Ginsenoside Rg1                    | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 27 | 33.16                | [M-H]⁻             | 408.9890       | 408.9871     | 4.6  | C₁₄H₁₀O₁₁S  | 409.99           | 3,3′-Di-O-methyllumic acid sulfate | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 28 | 34.34                | [M + FA-H]⁻       | 493.2308       | 493.2291     | 3.5  | C₂₁H₃₆O₁₀   | 448.23           | Geraniol 1-O-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 29 | 35.30                | [M-H]⁻             | 423.0051       | 423.0028     | 5.5  | C₁₇H₁₂O₁₁S  | 424.01           | 2,3,8-Tri-O-methyllumic acid sulfate | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
| 30 | 38.58                | [M-H]⁻             | 983.4580       | 983.4493     | 8.8  | C₄₈H₇₂O₂₁  | 984.46           | Licorice saponin A3                | 338.1386; 322.0389; 549.1654; 255.0655; 135.0104; 977.5391; 931.5254; 799.4847; 637.4346; 314.1417; |
In summary, these data indicate that JPQCD can reduce the inflammatory response by alleviating endoplasmic reticulum stress and regulating the PERK/eIF2α/ATF4/CHOP signaling pathway, with the specific mechanism shown in Figure 8.

4. Discussion

UC is a chronic recurrent intestinal inflammatory disease classified as one of the refractory diseases by the World Health Organization. Due to the many adverse effects of current therapies, it is urgent to find an effective and safe treatment method for UC. The purpose of this study was to investigate the efficacy and potential mechanism of JPQCD in reducing piroxicam-induced chronic colitis in IL-10−/− mice. We found that JPQCD repaired the intestinal mucosal barrier in mice, significantly reduced the piroxicam-induced colon inflammatory response, alleviated ER stress of colonic epithelial cells, and regulated the PERK/eIF2α/ATF4/CHOP pathway.

In our study, we used a chronic colitis model of IL-10−/− mice induced by piroxicam. The IL-10−/− mice is a genetic engineering model widely used for analyzing the causes of inflammatory bowel disease, which was established by Kühn et al. in 1993 [18]. In previous studies, we have demonstrated the efficacy of JPQCD in DSS-induced colitis in mice [13–15]. However, the use of chemical reagents to damage the colonic mucosal barrier of mice, increase its
permeability, and then trigger the production of inflammatory lesions in the intestinal tract is significantly different from the mechanism of inflammatory bowel disease induced by diet, immunity, infection, spirit, and other factors in human UC, which limits the experimental research. ©_he use of gene knockout technology to replicate animal models of UC has the characteristics of spontaneity and can simulate human UC, which is important for revealing the etiology, clarifying the genetic pathogenesis of the disease, and determining susceptibility genes [19]. It has been shown that, in C57BL/6 IL-10−/− mice, spontaneous colitis development is slow, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as piroxicam can induce moderately severe colitis in 2 wk. Acute symptoms turned chronic after withdrawal of the drug, and its pathological features were consistent with those observed in the colonic tissues of spontaneous colitis mice, while wild-type mice exposed to NSAIDs did not develop colitis [20, 21]. ©_he histopathological characteristics of IL-10−/− mouse colitis are similar to those of human IBD, including lamellar and submucosal

Figure 1: UPLC-Q-TOF/MS of JPQCD. (a) UPLC-HRMS base peak ion flow graph (BPC) negative ion mode for JPQCD; (b) UPLC-HRMS BPC-positive ion mode for JPQCD; (c) UPLC UV xhromatogram of JPQCD-UV 254 nm.
inflammatory cell infiltration, epithelial hyperplasia, crypt abscess, ulceration, and intestinal wall thickening [22, 23]. Compared with the traditional chemical reagents induction method, this model involves the interaction of genetic factors and immunity and can better simulate the multifactorial human IBD, which is of importance for exploring the pathogenesis of this disease [24]. In this study, piroxicam was added to the model feed at 200 ppm for 10 d to induce colitis in IL-10−/− mice, and the mice in the model group showed significant weight loss, thin fecal matter, and positive fecal occult blood test [16]. H&E staining showed that the colonic epithelium of the model

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Figure 2: JPQCD can improve the symptoms of experimental chronic colitis in IL-10−/− mice: (a) animal experiment design. IL-10−/− mice were induced by piroxicam for 10 days. Wild-type/control group/model group and JPQCD group were given normal saline or JPQCD daily. n = 6–8. (b) The body weight was measured every day. (c) Schematic diagram of disease activity index score and fecal occult blood test. (d) Colon length and statistics. Data are shown as the mean ± SEM. Compared to the model group, * P < 0.05, ** P < 0.01, *** P < 0.001.
Figure 3: Histologic images of mice colon (H&E, magnification ×100). (a) Wild-type group; (b) the control group; (c) model group; (d) low-dose JPQCD group; (e) middle-dose JPQCD group; (f) high-dose JPQCD group. The arrow represents the infiltration of inflammatory cells.

Figure 4: Transmission electron microscopy of mouse intestinal epithelial cells (magnification ×6000). (a) Wild-type group; (b) the control group; (c) model group; (d) low-dose JPQCD group; (e) middle-dose JPQCD group; (f) high-dose JPQCD group. Nu: nucleus; Mi: mitochondrial; ER: endoplasmic reticulum; rER: rough endoplasmic reticulum; Mv: microvillus.
**Figure 5:** Effects of Jianpi Qingchang decoction (JPQCD) on the expression of GRP78, PERK, eIF2α, ATF4, and CHOP mRNA by RT-PCR in piroxicam-induced colitis IL-10−/− mice. Data are shown as the mean ± SEM. Compared to the model group, *P < 0.05, **P < 0.01, ***P < 0.001.

**Figure 6:** Continued.
Figure 6: (a) Effects of Jianpi Qingchang decoction (JPQCD) on the expression of PERK/eIF2α/ATF4/CHOP pathway proteins assessed by western blot in piroxicam-induced colitis IL-10−/− mice. (b-f) Densitometric analysis was performed to determine each protein. β-Actin was used as the loading control. Data are shown as the mean ± SEM. Compared to the model group, *P < 0.05, **P < 0.01, ***P < 0.001. (g) Immunofluorescence staining for CHOP in colon tissues (magnification ×100).
group showed obvious ulceration, disordered arrangement of glands, and a large amount of inflammatory cell infiltration, indicating the success of the model.

IEC has an abundant ER structure and is continuously stimulated by intestinal flora, mucosal inflammatory mediators, and other ER stressors [25]. Studies have shown that excessive ER stress in IECs may be involved in the pathogenesis of IBD [26]. Increased expression of ER stress markers has also been observed in colonic epithelial tissues of active IBD patients [27, 28]. Therefore, improving ER stress to restore intestinal homeostasis may be a potential therapeutic target for IBD. Therefore, improving ER stress to restore intestinal homeostasis may be a potential therapeutic target for IBD. PERK is a transcription factor downstream of PERK and a direct target of ATF4, and studies have confirmed that initiation of CHOP is induced by the continuous activation of the PERK pathway [30]. When a severe UPR is caused, ATF4 upregulates CHOP gene expression and induces apoptosis. In this study, the phosphorylation of PERK and eIF2α, the expression of ATF4 and CHOP proteins, and the mRNA expression of PERK, eIF2α, ATF4, and Chop in the colon tissue of the model group were increased, suggesting that the PERK-eIF2α-ATF4-CHOP signaling pathway was activated. JPQCD could significantly downregulate the phosphorylation of PERK and eIF2α, the expression of ATF4 and CHOP proteins, and CHOP mRNA levels.
expression of PERK, eIF2α, ATF4, and CHOP so as to inhibit intestinal inflammation. Consistent results were obtained with immunofluorescence staining for the apoptotic gene CHOP, and JPQCD significantly reduced the fluorescent expression of CHOP in colonic sections. These results suggest that JPQCD alleviates ER stress and plays an anti-inflammatory role by inhibiting the PERK/eIF2α/ATF4/CHOP pathway.

The imbalance between proinflammatory and anti-inflammatory cytokines in IBD affects local intestinal inflammation and tissue damage. Blocking these inflammatory mediators is one of the mechanisms that reduce or even reverse the symptoms of IBD [31]. ER stress increases the production of cytokines, including IL-1β, IL-6, IL-8, and TNF-α [32,33]. Studies have shown that CHOP can induce dendritic cells to secrete IL-23, which may promote the production of IL-17 by local T cells, thus triggering an innate immune response [34]. Under the condition of chronic inflammation, the expression of ER stress marker molecules GRP78 and p-eIF2α was increased in the intestinal epithelial cells of IL-10−/− mice [35]. Therefore, we hypothesized that IL-10−/− mice exposed to piroxicam produced a large number of inflammatory cytokines in the colon and damaged the intestinal mucosa. The barrier function of the intestinal mucosa is damaged, leading to excessive ER stress, causing intestinal inflammation, which in turn promotes cytokine secretion and further aggravates inflammation. IL-17 is a proinflammatory cytokine specifically secreted by helper T cells (Th17). When IL-17 specifically binds to IL-17R on the cell surface, it activates IkB kinase, resulting in the phosphorylation of IkB protein, ubiquitination and degradation, activation of NF-κB signaling pathway and activation of NLRP3 inflammasome [36]. Our results showed that the content of IL-17 and mRNA expression in colonic tissue of mice in the model group were significantly increased, confirming that IL-17 was involved in the piroxicam-induced experimental colitis in IL-10−/− mice, and the intervention of JPQCD could reduce IL-17 level. Previous studies have shown that JPQCD can reduce DSS-induced acute colitis in mice by inhibiting the activation of NF-κB [14]. In this study, the protein expression of NF-κB, p-NF-κB p65 and mRNA expression of NF-κB in the colon tissues of IL-10−/− induced by piroxicam were increased, while JPQCD significantly down-regulated the expression of NF-κB.

JPQCD is composed of nine TCMs, each of which contains a variety of compounds, including known anti-UC components, such as berberine, astragalus polysaccharide, and Codonopsis pilosula polysaccharide. Pharmacological studies showed that berberine, the active component of Coptis chinensis, could inhibit the Th17 response, protect the colon barrier function of mice, regulate the intestinal flora of mice, and have a significant protective effect on DSS-induced colon injury and inflammation in mice [37–39]. Astragalus polysaccharide can reduce the severity of DSS-induced colitis in mice, and this protective effect may be mediated by inhibiting the activation of NF-κB [40]. Codonopsis pilosula polysaccharide and Coptis chinensis saponin, the main active components of Codonopsis pilosula, can synergistically regulate the balance of proinflammatory and anti-inflammatory cytokines, enhance the immune response of the body, and inhibit the colonization of pathogenic bacteria, thereby reducing the symptoms of colitis in UC mice [41]. Polysaccharide from Scutellaria baicalensis Georgi can improve colitis by inhibiting the NF-κB signaling pathway and activation of NLRP3 inflammasome [42]. The synergistic effect of various compounds may be key to the role of JPQCD in the treatment of UC. In this study, JPQCD significantly improved UC symptoms, such as weight loss, increased DAI, and improved colonic shortening and colorectal bleeding in IL-10−/− mice with piroxicam-induced colitis. In addition, JPQCD inhibited crypt epithelial deformation, goblet cell loss, inflammatory cell infiltration, and inflammatory response and alleviated histopathological lesions in the colonic mucosa and submucosa of mice. These results suggest that JPQCD is an ideal natural drug for the prevention and treatment of UC and other IBDs.
In this study, the efficacy of JPQCD did not increase with the dose. In most cases, a medium-dose JPQCD is more effective. Due to the complex components and effects of traditional Chinese medicine and the individual differences of animals and the limited number of samples, there is often no obvious dose-effect relationship in pharmacodynamics tests. The medium dose is the equivalent dose, which is based on the experience of JPQCD and the experience of famous experts. This animal experiment has well verified the clinical efficacy of the drug.

5. Conclusion

In summary, IEC stress induced by piroxicam in IL-10−/− mice is excessive and activates the PERK/eIF2α/ATF4/CHOP pathway. JPQCD can reduce ER stress, regulate the PERK/eIF2α/ATF4/CHOP pathway, repair the intestinal mucosal barrier in mice, and prevent and treat UC.

Abbreviations

ER: Endoplasmic reticulum
IEC: Intestinal epithelial cells
UC: Ulcerative colitis
JPQCD: Jianpi Qingchang decoction
RT-PCR: Reverse transcription-polymerase chain reaction
ELISA: Enzyme-linked immunosorbent assay
IBD: Inflammatory bowel disease
TCM: Traditional Chinese medicine
UPR: Unfolded protein response
PERK: Protein kinase-related endoplasmic reticulum kinase
IRE1: Inositol-requiring enzyme 1
ATF6: Activating transcription factor 6
GRP78: Glucose-regulated protein 78
DSS: Dextran sodium sulfate
NF-xB: Nuclear factor-xB
IL-1β: Interleukin-1β
DAI: Disease activity index
H&E: Hematoxylin and eosin
NSAIDS: Nonsteroidal anti-inflammatory drugs.

Data Availability

The datasets generated during and/or analyzed during the current study will be available upon request from the principle investigator. The shared data will only be allowed to be used by the applicant for scientific studies. No commercial activities are allowed.

Ethical Approval

This study was approved by the Laboratory Animal Ethics Committee of Shanghai University of Chinese Medicine (PZSHUTCM190912020).

Consent

All participants signed informed consent.

Disclosure

Chen Q and Zhang YL are co-first authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this work.

Authors’ Contributions

Chen Q and Zhang YL contributed equally and finished the major experiments; Dai YC performed the experiments and analyzed the data; Zhang ZW, Chen YJ, and Tang YJ participated in the treatment of animals; Qiao D supplemented some molecular experiments. Tang ZP designed and coordinated the research; all authors read and approved the final manuscript. Qian Chen and Ya-Li Zhang contributed equally to this work.

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