

Qilian Jiechang Ning Alleviates TNBS-Induced Ulcerative Colitis in Mice and *Segatella copri* Outer Membrane Vesicle-Triggered Inflammation in Colon Epithelial Cells via the Caspase-1/11-GSDMD Pathways

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Highlights

- The outer membrane vesicles (OMVs) of *Segatella copri* (*S. copri*) induce inflammatory responses and dysregulation of tight junction protein expression in colon epithelial cells.
- Qilian Jiechang Ning reduces the inflammatory responses induced by *S. copri* OMVs in colon epithelial cells.
- Qilian Jiechang Ning alleviates ulcerative colitis by inhibiting Caspase-1 and Caspas-11-mediated inflammation.

Keywords

Ulcerative colitis · Qilian Jiechang Ning · *Segatella copri* · Outer membrane vesicles · Inflammatory response

Abstract

Introduction: Qilian Jiechang Ning (QJN), a traditional Chinese herbal formula, has demonstrated potential therapeutic effects in the treatment of ulcerative colitis (UC). This study aims to investigate the mechanism of QJN in the outer membrane vesicles (OMVs) of *Segatella copri* (*S. copri*)-induced colon epithelial cells and UC mice. **Methods:** Transmission electron microscopy (TEM) and nanoparticle

tracking analysis (NTA) were utilized to assess the morphology and size of OMVs. Inflammation markers and tight junction protein levels in HCoEpiCs induced by OMVs were monitored using ELISA and western blot. QJN was administered to intervene in HCoEpiCs treated with *S. copri* OMVs. Additionally, trinitrobenzene sulfonic acid (TNBS)-induced mouse models were conducted to evaluate the therapeutic effects of QJN on UC. **Results:** *S. copri* OMVs treated with QJN demonstrated a significant reduction in particle size, protein concentration, and LPS content. In HCoEpiCs, QJN effectively decreased the expression of inflammation-inducing cytokines (IL-1 β , IL-18, IL-6, TNF- α) and proinflammatory proteins (GSDMD-N, NLRP3, ASC, cleaved Caspase-1,

cleaved Caspase-4) triggered by *S. copri* OMVs, while enhancing the expression of tight junction proteins (ZO-1 and Occludin). In the UC mouse models, QJN significantly reduced the Disease Activity Index (DAI), improved colon length, lowered LPS levels, ameliorated colonic tissue damage, and inhibited Caspase-1- and Caspase-11-dependent inflammatory responses. **Conclusion:** QJN can alleviate *S. copri*-OMV-induced inflammatory response in colonic epithelial cells and reduce symptoms of UC in mouse models by modulating the Caspase-1 and Caspase-11 pathways.

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Introduction

Ulcerative colitis (UC) is a prevalent idiopathic inflammatory bowel disease that primarily affects the mucosa and submucosa of the colon and rectum, leading to various gastrointestinal complications, including carcinogenesis [1, 2]. Furthermore, the incidence of UC is increasing globally each year. The exact etiology of UC remains poorly understood, involving complex interactions among genetic susceptibility, environmental factors, dysbiosis of gut microbiota, and abnormalities in the immune system [3]. Due to the unclear pathogenesis of UC, no definitive treatment exists; clinical management typically focuses on protecting the intestinal mucosa, administering anti-inflammatory therapies, and eradicating pathogens. To date, the primary medications for treating UC include 5-aminosalicylic acid, immunosuppressants, antibiotics, and corticosteroids [4]. While most UC patients can effectively manage their condition with these drugs, 20%–30% of patients may still experience various complications, ineffective treatments, or steroid dependency due to secondary loss of response and the toxic side effects of some medications. In particular, long-term use of immunosuppressants increases the risk of infections, corticosteroids can lead to osteoporosis and metabolic abnormalities, and biological therapies may encounter issues of initial nonresponse and secondary resistance [5–7]. Therefore, exploring and clarifying effective alternative treatment strategies for UC has become crucial.

Qilian Jiechang Ning (QJN) is a traditional Chinese herbal formulation used to treat UC and consists of 14 herbal ingredients, including *Astragalus membranaceus* (*A. membranaceus*), *Coptis chinensis* (*C. chinensis*), *Panax ginseng* (*P. ginseng*), and others. Preliminary

preclinical evidence suggests that *A. membranaceus* may be a potential candidate for the treatment of inflammatory bowel diseases. The active components in *A. membranaceus*, such as astragalosides and polysaccharides, have demonstrated a range of pharmacological effects, including anti-inflammatory, antioxidant, and immunomodulatory activities [6]. Additionally, astragalosides and polysaccharides have been shown to alleviate experimental UC induced by dextran sulfate sodium [8, 9]. *C. chinensis* can alleviate intestinal barrier damage caused by UC through its anti-inflammatory effects and regulation of gut microbiome balance [10]. Both *C. chinensis* polysaccharides and berberine also provide protection against UC [11]. Ginsenosides derived from *P. ginseng* can ameliorate UC by inhibiting intestinal inflammation and barrier damage [12]. Our previous study found that QJN can reduce levels of inflammatory factors and alleviate pathological damage in the rat colon [13] (in Chinese). However, the specific mechanism by which QJN treats UC remains unclear.

UC is primarily driven by the activation of the Caspase-1 pathway during typical inflammation and the Caspase-11 pathway during nonclassical inflammation [14, 15]. Caspase-1 and Caspase-11 facilitate the production of cytokines in colon tissue and mouse colonic mucosal epithelial cells, thereby exacerbating colonic injury in UC [16]. Caspase-1 is a key component of the pyrin domain-containing 3 (NLRP3) inflammasome; its activation promotes the maturation and secretion of inflammatory mediators such as interleukin (IL)-1 β and IL-18 in UC [17]. Caspase-11 is activated in response to intracellular bacterial infections by detecting endogenous lipopolysaccharides (LPS), which subsequently triggers pyroptosis [18, 19].

Patients with UC exhibit distinct gut microbial characteristics compared to healthy individuals. Gut bacteria play a crucial role in regulating the host's physiological state by secreting outer membrane vesicles (OMVs) [20]. OMVs are nanometer-sized vesicles produced by Gram-negative bacteria that deliver LPS into the cytosol, triggering Caspase-11-dependent effector responses [18]. In inflammatory bowel diseases, OMVs can initiate inflammatory signaling by exposing surface LPS, which mediates inflammasome formation and the secretion of inflammatory factors [21, 22].

Previous research has indicated that the abundance of *Prevotella* significantly increases in animal models of UC, while QJN treatment substantially reduces its abundance [13] (in Chinese). This suggests that QJN may regulate the progression of UC through the modulation of *Prevotella*. The increase in *Prevotella* plays a proinflammatory role by

activating Toll-like receptor 4 (TLR-4) through LPS production, which results in abdominal pain [23]. *Segatella copri* (*S. copri*, formerly known as *Prevotella copri*) is the most abundant species within the *Prevotella* genus that inhabits the human large intestine [23]. Additionally, *S. copri* can stimulate Th17 responses and promote inflammation through specific strain-dependent features [24]. *S. copri*-derived LPS can activate nuclear factor κ B and NLRP3 inflammasome signaling pathways [25]. Therefore, this study aims to clarify whether *S. copri*-derived OMVs mediate inflammation through LPS in colonic epithelial cells. Furthermore, it seeks to explore the mechanism by which QJN alleviates Caspase-1- and Caspase-11-dependent inflammatory responses in UC mouse models and *S. copri* OMV-induced cell models.

Methods

QJN Preparation

A. membranaceus (15 g), *P. ginseng* (15 g), *Rhizoma Atractylodis macrocephalae* (15 g), *Poria cocos* (15 g), *Pericarpium Citri reticulatae* (10 g), *C. chinensis* (10 g), Dried ginger (*Zingiber officinale* Roscoe, 6 g), *Cattail pollen* (10 g), Dandelion (*Taraxacum officinale*, 15 g), *Herba Patriniae* (15 g), Root Bark of *Ailanthus excelsa* Roxb (10 g), *Aucklandiae Radix* (10 g), White peony (*Paeoniae Radix Alba*, 15 g), and Liquorice (*Glycyrrhiza glabra*, 5 g) were all purchased from the Hunan Provincial Hospital of Integrated Traditional Chinese and Western Medicine (Changsha, China). The herbs are soaked in distilled water for 40 min. After the initial boiling over high heat, the mixture is simmered on low heat for 30 min. Following a second boiling over high heat, it is again simmered on low heat for 20 min. Finally, the two decoctions are combined and concentrated to yield a medicinal liquid with a concentration of 2.48 g/mL of raw material.

Bacteria and Cell Strains

S. copri (DSM18205) from human feces were obtained from BIOBW (Beijing, China). *S. copri* were cultured on PYG agar supplemented with 5% (v/v) sterile defibrinated sheep blood and incubated at 37°C for 2–7 days in an anaerobic workstation filled with a gas mixture of 80% N₂, 10% CO₂, and 10% H₂ [26]. HCoEpiC cells (Nanjing Saihongrui Biotechnology Co., Ltd., Nanjing, China) were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, streptomycin (100 mg/mL), penicillin (100 U/mL), and 1% nonessential amino acids at 37°C with 5% CO₂.

Extraction and Identification of OMVs

S. copri were divided into two groups: a control group (normal culture) and a QJN group (cultured with 1.27 g/mL QJN). Following centrifugation at 15,000 g for 30 min, the supernatant was filtered at 4°C using 0.45- μ m filters to eliminate any bacterial precipitates. Next, the OMV supernatant was washed once with sterile PBS and concentrated to 1 mL by centrifugation at 170,000 g for 60 min at 4°C. The concentrated solution was then filtered through 0.45- μ m filters and stored at –80°C for later use. OMVs in the control group are referred to as *S. copri* OMVs, and OMVs in the QJN group are referred to as QJN OMVs. The morphology of OMVs was observed using a transmission electron microscope (TEM) with a negative staining method. The average size of the OMVs was measured through nanoparticle tracking analysis (NTA). The protein content of the purified OMVs was quantified using a BCA Protein Assay Kit (Beyotime, Shanghai, China). The concentration of LPS in the OMVs was detected using the limulus amoebocyte lysate assay with the Endotoxin Assay Kit (C0276S, Beyotime) [27].

Grouping of HCoEpiC

To investigate the role of *S. copri* OMVs in inflammatory responses and the dysregulation of tight junction protein expression, HCoEpiCs were divided into three groups: a control group (normal culture), an LPS group (1 μ g/mL for 24 h), and an *S. copri* OMV group (1 μ g/mL for 24 h). To assess the effect of QJN on inflammation and tight junction protein expression, HCoEpiCs were further divided into four groups: a control group, an *S. copri* OMV group (1 μ g/mL for 24 h), an *S. copri* OMVs plus QJN group (1 μ g/mL *S. copri* OMVs and 1.27 g/mL QJN for 24 h), and a QJN OMV group (1 μ g/mL for 24 h).

Establishment and Treatment of UC

Six-week-old male Balb/c mice (weighing between 18 and 25 g) were purchased from Vital River (Beijing, China). After 1 week of acclimatization, the mice were randomly divided into four groups (control, TNBS, TNBS plus QJN, TNBS plus Mesalazine; $n = 6$ per group) using a random number table method. To induce UC, the mice were anesthetized with an intraperitoneal injection of 10% chloral hydrate (4 mL/kg). A slow injection of 3% TNBS (50 mg/kg) and 50% ethanol (0.25 mL) was administered into the rectum using a 0.4-mm diameter polyethylene catheter lubricated with paraffin oil (approximately 8 cm of the catheter was inserted into the rectum). Normal control mice received an injection of normal saline in the colon [17]. For the treatment of UC, mice were administered QJN (2.48 g/kg) or Mesalazine

(0.067 g/kg) via gavage after 7 days of modeling (2 mL, once daily, for a continuous treatment period of 21 days). The control group and the TNBS group received 2 mL of normal saline via gavage simultaneously. This study protocol was reviewed and approved by the Ethics Committee of The Affiliated Hospital of Hunan Academy of Traditional Chinese Medicine (Approval No. SBWJW2024-0008).

UC Assessment

Daily monitoring of body weight, stool consistency, and the presence of blood in feces enabled the assessment of UC severity in each mouse. We calculated the Disease Activity Index (DAI) using a standardized scoring system that included: (a) weight loss scoring: 0 for no loss, 1 for a reduction of 1%–5%, 2 for 5%–10%, 3 for 10%–20%, and 4 for a loss exceeding 20%; (b) diarrhea assessment: 0 for normal stool, 2 for loose stool, and 4 for watery diarrhea; and (c) rectal bleeding evaluation: 0 for absence of blood, 2 for slight bleeding, and 4 for significant bleeding [28]. At the end of the 21-day experimental period, the mice were euthanized for sample collection. Following euthanasia, blood samples were collected, and colonic tissues were excised. The length of the colon was then measured as a morphological marker of UC progression.

Histological Analysis

The distal colons were meticulously processed, which included fixation, embedding, and sectioning, prior to undergoing hematoxylin and eosin staining. A standardized scoring system was utilized for histopathological evaluation, encompassing various parameters. For assessing inflammation severity, a scale from 0 (none) to 3 (severe) was implemented. The depth of the observed lesions was evaluated on a scale from 0 (absence) to 4 (transmural). Crypt damage was quantified by assigning scores ranging from 0 (none) to 4 (complete loss of crypt structure and epithelium). The extent of the lesions was assessed on a scale from 1 (1%–25% involvement) to 4 (76%–100% involvement) [28].

Enzyme-Linked Immunosorbent Assay

Blood samples were centrifuged (3,000 rpm, 15 min at 4°C) and the serum was carefully collected. The levels of IL-1 β (E-EL-H0149 and E-EL-M0037; Elabscience, Wuhan, China), IL-18 (E-EL-H0253 and E-EL-M0730; Elabscience), IL-6 (E-EL-H6156 and E-EL-M0044; Elabscience), tumor necrosis factor- α (TNF- α) (E-EL-H0109 and E-EL-M3063; Elabscience), and LPS (JL20691; Jianglai Biological, Shanghai, China) in the cell supernatant or serum were quantified using enzyme-

linked immunosorbent assay (ELISA) kits. Samples (100 μ L per well) were added to ELISA plates and incubated for 90 min at 37°C. After removing the liquid, a biotinylated antibody working solution (100 μ L per well) was added for a 1-h incubation. Following washing, 100 μ L of HRP-conjugate working solution was added for a 30-min incubation, after which 90 μ L of substrate reagent was added and incubated for 15 min at 37°C. Subsequently, 50 μ L of stop solution was added, and absorbance was measured at 450 nm using a microplate reader (Bio-Rad). For LPS detection, 50 μ L of samples and HRP-antigen working solution were added to each well and incubated at 37°C for 1 h. The remaining steps were performed as described above.

Immunofluorescence

Each mouse's distal colon was fixed in 4% formaldehyde, embedded in O.C.T. compound, and rapidly frozen in liquid nitrogen before sectioning. Eight-micrometer-thick frozen sections of the distal colons were prepared for immunofluorescence assays. Initially, the sections were thoroughly washed with cold phosphate-buffered saline and then blocked with 0.3% bovine serum albumin for 1 h at room temperature. Following this, the sections were incubated overnight with mouse anti-Caspase-1 (RRID: AB_781816; sc-56036, 1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or mouse anti-Caspase-11 (RRID: AB_10988215; sc-374615, 1:50, Santa Cruz Biotechnology) at 4°C. The next day, the sections were labeled with rhodamine-conjugated secondary antibodies (Goat Anti-Mouse IgG H&L, RRID: AB_2576208, ab150113, 1:500, Abcam; RRID: AB_2687948, ab150115, 1:500, Abcam) for 2 h at room temperature. Finally, the resulting images were meticulously examined using an Olympus IX73 microscope (Tokyo, Japan).

Western Blotting

Proteins from cultured cells or distal colon tissues were extracted using the radioimmunoprecipitation assay lysis buffer, which contains protease inhibitors to maintain protein integrity. The quality of the total protein sample was assessed using the BCA Protein Assay Kit (Beyotime) according to the manufacturer's instructions. The extracted protein was boiled for 10 min and then subjected to 10% sodium dodecyl sulfate-polyacrylamide gel for separation. Subsequently, the separated proteins were transferred onto polyvinylidene fluoride membranes. After blocking the membranes with 5% skim milk in Tris-buffered saline with Tween-20 for 2 h at room temperature, the membranes were cut according to the

molecular weight of the target proteins and then incubated with specific primary antibodies: rabbit anti-GSDMD-N (RRID: AB_3076218; DF13758, 1:500, Affinity Biosciences, Liyang, Jiangsu, China; RRID: AB_2916166; ab215203, 1:1,000, Abcam, Cambridge, MA, USA), rabbit anti-NLRP3 (RRID: AB_2889890; ab263899, 1:1,000, Abcam), rabbit anti-ASC (RRID: AB_2838270; DF6304, 1:1,000, Affinity Biosciences), rabbit anti-cleaved Caspase-1 (RRID: AB_2845463; AF4005, 1:500, Affinity Biosciences), rabbit anti-cleaved Caspase-4/11 (RRID: AB_2923217; ab180673, 1:1,000, Abcam; RRID: AB_2837858; AF5373, 1:500, Affinity Biosciences), rabbit anti-ZO-1 (RRID: AB_10680012; ab96587, 1:1,000, Abcam), rabbit anti-Occludin (RRID: AB_2737295; ab216327, 1:1,000, Abcam), and rabbit anti-GAPDH (RRID: AB_307275; ab9485, 1:2,500, Abcam) overnight at 4°C. In the following day, the membranes were washed three times with Tris-buffered saline containing Tween-20 and then incubated with Goat Anti-Rabbit IgG H&L (HRP) (RRID: AB_955447; ab6721, 1:10,000, Abcam) for 2 h at room temperature. After incubation, the protein bands were visualized using a chemiluminescence imaging system. Protein expressions were analyzed using ImageJ software.

Statistical Analysis

All data were analyzed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Sample size determination was conducted using G*Power software (version 3.1.9.7, Heinrich-Heine-Universität Düsseldorf, Germany) with an alpha level (α) of 0.05 and a desired power ($1-\beta$) ≥ 0.80 . For in vitro experiments, three independent biological replicates ($n = 3$) were performed, with technical triplicates for each experiment. In the in vivo studies, 6 mice per group ($n = 6$) were utilized based on power analysis calculations. Statistical differences were assessed using one-way analysis of variance for multiple groups or Student's *t* test for comparisons between two groups. *p* values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

Extraction and Characterization of OMVs

This study is divided into two groups: a control group (*S. copri* OMVs) and a QJN group (QJN OMVs), each focusing on the extraction and identification of OMVs. TEM revealed that the OMVs secreted by both the control and QJN groups exhibited either an ellipsoidal structure or a spherical vesicular structure, with no significant

morphological differences between *S. copri* OMVs and QJN OMVs (Fig. 1a). The average particle diameter of *S. copri* OMVs was approximately 118.2 nm, while QJN OMVs had a significantly smaller average diameter of about 81.5 nm (Fig. 1b). A BCA protein assay indicated that the protein concentration of QJN OMVs was significantly lower compared to that of *S. copri* OMVs (Fig. 1c). The limulus amoebocyte lysate test was conducted to assess the LPS content, revealing that QJN OMVs contained significantly less LPS than *S. copri* OMVs (Fig. 1d). Collectively, these results demonstrate that *S. copri* OMVs were successfully obtained.

S. copri OMVs Induce Inflammatory Responses and Dysregulation of Tight Junction Protein Expression in Colonic Epithelial Cells

To verify the inflammatory responses induced by *S. copri* OMVs, HCoEpiCs were treated with LPS or *S. copri* OMVs. ELISA was employed to measure the expression levels of IL-1 β , IL-18, IL-6, and TNF- α . The results demonstrated that *S. copri* OMVs significantly enhanced the production of these cytokines (Fig. 2a). Additionally, Western blot analysis was conducted to assess the expression of pyroptosis-associated proteins and tight junction proteins. This analysis revealed that *S. copri* OMVs significantly upregulated the expression of GSDMD-N, NLRP3, ASC, cleaved Caspase-1, and cleaved Caspase-4, while downregulating the expression of ZO-1 and Occludin (Fig. 2b). These findings indicate that *S. copri* OMVs can induce inflammation mediated by Caspase-1 and Caspase-4 in colonic epithelial cells, as well as disrupt the expression of tight junction proteins.

QJN Alleviated Inflammatory Responses and Enhanced the Expression of Tight Junction Proteins in Colonic Epithelial Cells

ELISA was employed to assess inflammatory factors, revealing a significant decrease in IL-1 β , IL-18, IL-6, and TNF- α levels in colonic epithelial cells induced by *S. copri* OMVs following QJN treatment. Furthermore, the capacity of QJN OMVs to upregulate the levels of IL-1 β , IL-18, IL-6, and TNF- α was notably weaker compared to *S. copri* OMVs (Fig. 3a). Western blot analysis of pyroptosis-related proteins and tight junction protein expression demonstrated that QJN significantly downregulated the expression of GSDMD-N, NLRP3, ASC, cleaved Caspase-1, and cleaved Caspase-4 induced by *S. copri* OMVs in colonic epithelial cells, while simultaneously upregulating ZO-1 and Occludin. The impact of QJN OMVs on these proteins was less pronounced than that of *S. copri* OMVs (Fig. 3b). These results suggest that

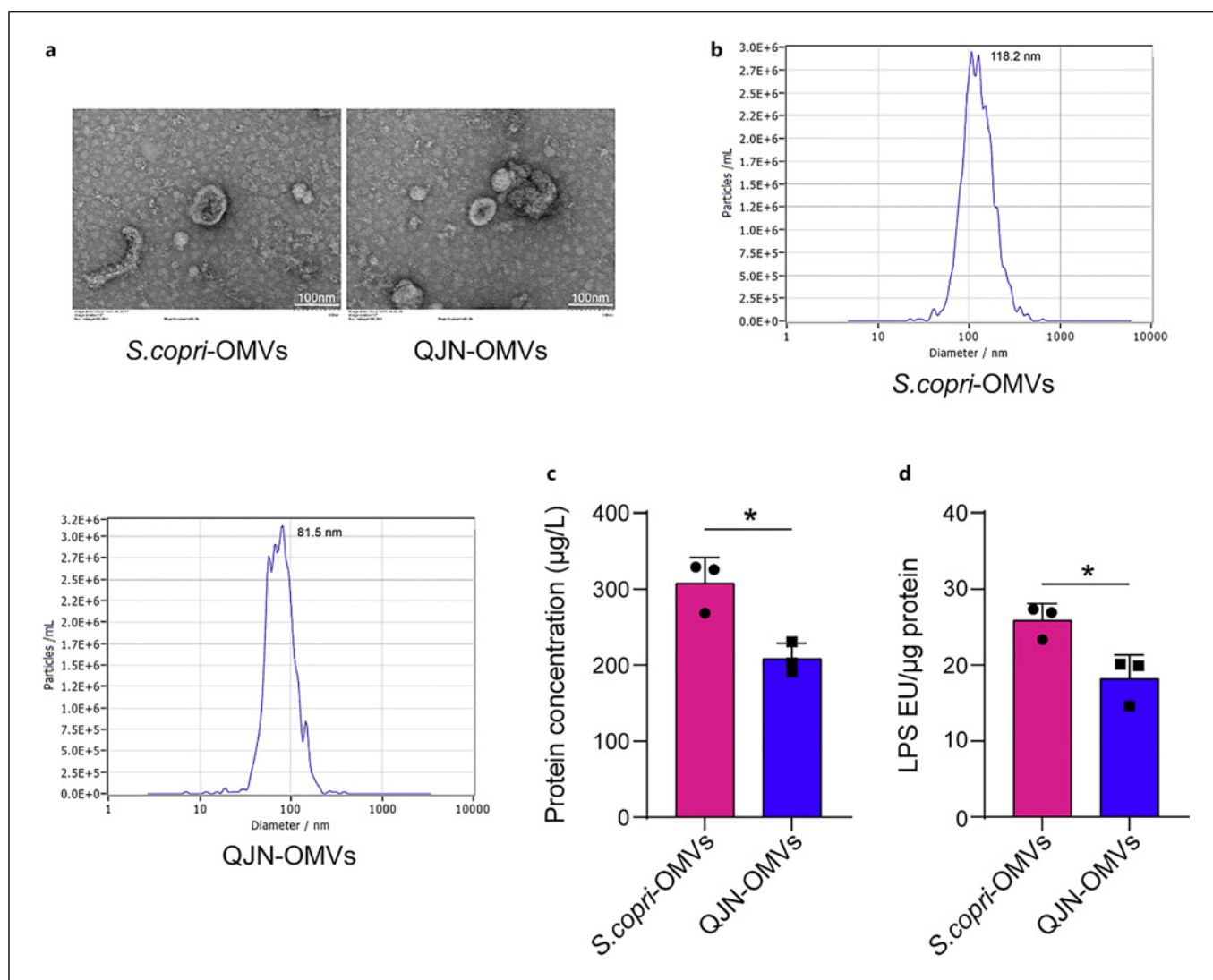


Fig. 1. Extraction and identification of OMVs. **a** OMV morphology was observed using transmission electron microscopy. **b** OMV size distribution was detected by NTA. **c** The protein concentrations of OMVs were measured by a BCA protein assay kit. **d** LPS content of purified OMVs was determined by the LAL assay. Statistical differences between the QJN OMV group and the *S. copri* OMV group were assessed using Student's *t* test. *n* = 3. **p* < 0.05. LAL, limulus amoebocyte lysate.

QJN modulates inflammatory responses and tight junction protein expression in colonic epithelial cells through *S. copri* OMVs.

QJN Ameliorates Symptoms of TNBS-Induced UC

To evaluate the ameliorative effects of QJN on TNBS-induced UC, 6-week-old male Balb/c mice were subjected to TNBS modeling. Seven days post-modeling, the mice were treated with QJN (2.48 g/kg) or Mesalazine (0.067 g/kg) for a duration of 21 days. Compared to the control group, the body weight of the TNBS-induced

group significantly reduced; however, treatment with QJN and Mesalazine significantly improved this weight loss (Fig. 4a). The DAI scores of the TNBS-induced group were significantly elevated compared to the control group, while both QJN and Mesalazine significantly reduced the DAI scores (Fig. 4b). Colon photography measurements revealed that TNBS treatment resulted in a shortened colon length in the mice, whereas treatment with QJN or Mesalazine increased colon length (Fig. 4c). ELISA detection of serum LPS levels indicated that TNBS significantly elevated these levels, which were subsequently

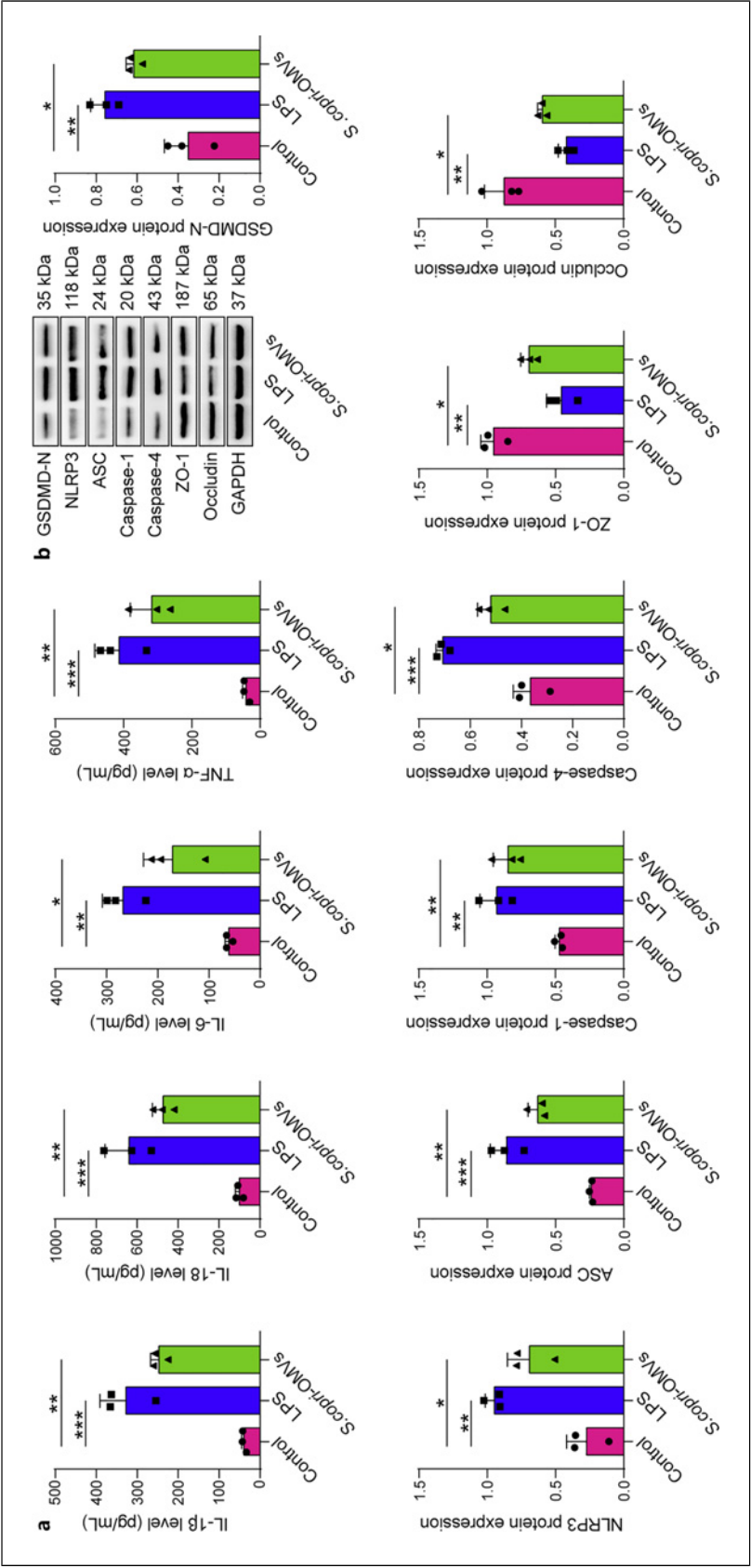


Fig. 2. *S. copri* OMVs induce inflammation and dysregulation of tight junction protein expression in colonic epithelial cells. **a** ELISA detection of IL-1 β , IL-18, IL-6, and TNF- α expression. **b** Western blot analysis of protein levels of GSDMD-N, NLRP3, ASC, cleaved Caspase-1, ZO-1, and Occludin in cell lysates. The presented blots were derived from the same gels. Colonic epithelial cells were cultured in standard conditions and treated with 1 μ g/mL of *S. copri* OMVs or LPS for 24 h. Statistical differences among groups were assessed using one-way analysis of variance (ANOVA), with comparisons made among the *S. copri* OMVs or LPS-treated group and the untreated control group. $n = 3$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

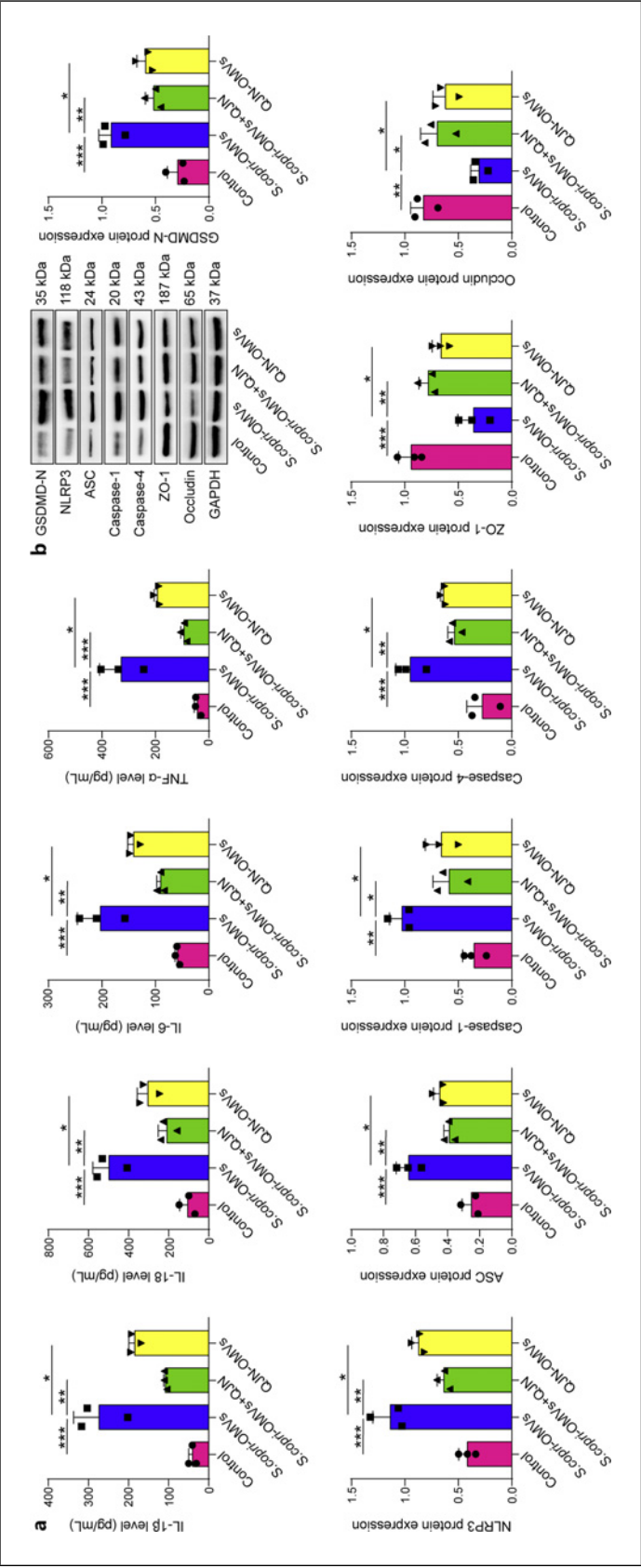


Fig. 3. QJN modulates inflammatory responses and tight junction protein expression in colonic epithelial cells. **a** ELISA detection of IL-1β, IL-18, IL-6, and TNF-α expression. **b** Western blot analysis of protein levels of GSDMD-N, NLRP3, ASC, cleaved Caspase-1, ZO-1, and Occludin. The presented blots were derived from the same gels. Colonic epithelial cells were cultured in standard conditions and treated with 1 μg/mL of *S. copri* OMVs and/or 1.27 g/mL of QJN, or 1 μg/mL of QJN OMVs for 24 h. Statistical differences among groups were assessed using one-way ANOVA. *S. copri* OMV group vs. control group, QJN OMV group and *S. copri* OMV plus QJN group vs. *S. copri* OMV group. *n* = 3. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

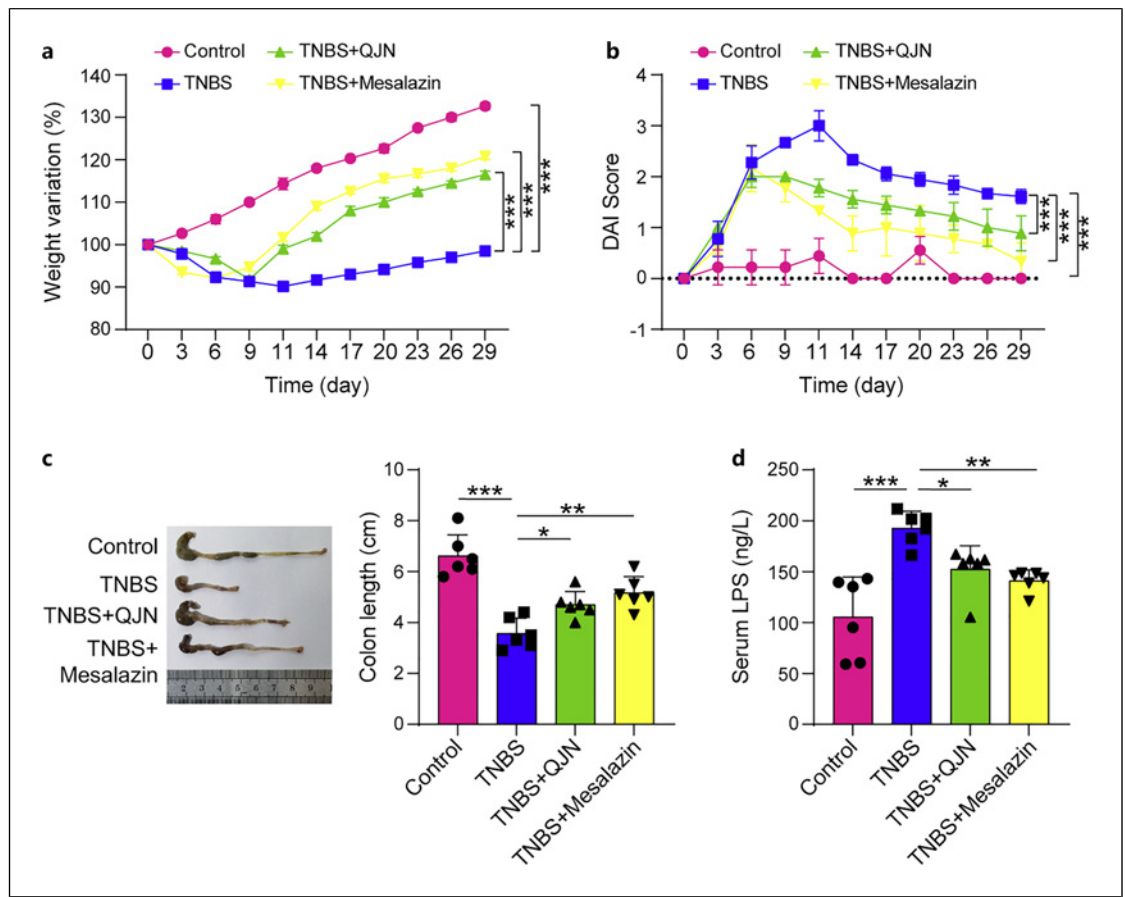


Fig. 4. QJN improves TNBS-induced UC symptoms. **a** Statistical analysis of body weight changes. **b** Disease Activity Index (DAI) scoring. **c** Colon photography and length measurement. **d** ELISA detection of serum LPS levels. The mice were divided into four groups: control, TNBS, TNBS plus QJN, and TNBS plus Mesalazine. Statistical differences were assessed using one-way ANOVA. TNBS group vs. control group, TNBS plus QJN group, and TNBS plus mesalazine group vs. TNBS group. $n = 6$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

reduced by QJN or Mesalazine treatment (Fig. 4d). Histological analysis of mouse colonic tissues demonstrated that treatment with QJN or Mesalazine significantly alleviated inflammatory cell infiltration, edema, epithelial hyperplasia, and goblet cell dysfunction induced by TNBS (Fig. 5). These results indicate that QJN can effectively improve colonic damage in UC mice.

QJN Alleviates UC by Inhibiting Caspase-1- and Caspase-11-Mediated Inflammation

ELISA of serum samples from various mouse groups revealed that treatment with QJN or Mesalazine significantly reduced the levels of IL-1 β , IL-18, IL-6, and TNF- α in UC mice (Fig. 6a). Western blot analysis of colonic proteins from these mouse groups showed that QJN or Mesalazine significantly downregulated the expression of GSDMD-N, NLRP3, ASC, cleaved Caspase-1, and cleaved

Caspase-11 in the colons of UC mice, while upregulating ZO-1 and Occludin (Fig. 6b). Immunofluorescence detection of colonic levels of Caspase-1 and Caspase-11 showed that their expression was significantly higher in the TNBS group and notably lower in the QJN and Mesalazine treatment groups compared to the TNBS group (Fig. 6c). Taken together, these findings suggest that QJN can alleviate UC by inhibiting Caspase-1- and Caspase-11-mediated colonic inflammation.

Discussion

As the science of the microbiome advances, the intricate relationship between gut microbiota and host health is becoming increasingly evident [29]. Research indicates that the pathogenesis of UC may be closely

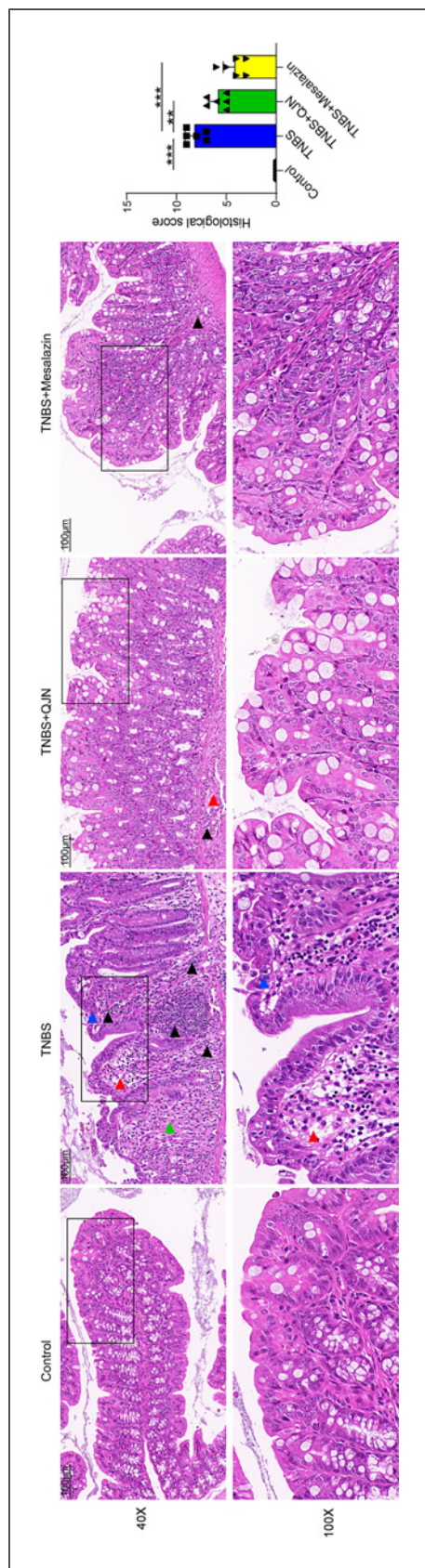


Fig. 5. QJN improves TNBS-induced colonic pathological damage. The mice were divided into four groups: control, TNBS, TNBS plus QJN, and TNBS plus Mesalazine. HE staining was conducted for observation of colonic pathological damage. Black arrow indicates inflammatory cell infiltration; red arrow indicates edema; green arrow indicates epithelial hyperplasia; and blue arrow indicates disordered arrangement of goblet cells. Statistical differences were assessed using one-way ANOVA. TNBS group vs. control group, $TNBS + QJN$ group, and $TNBS + mesalazine$ group vs. TNBS group. $n = 6$. $***p < 0.001$, $**p < 0.01$, $*p < 0.05$.

linked to alterations in specific bacterial species within the gut microbiome [30]. The abnormal proliferation of *S. copri* is frequently observed in UC patients, and it has been confirmed that its OMVs can disrupt the host's immune response [31–33]. The components secreted by OMVs may directly contribute to the disruption of the intestinal epithelial barrier and subsequent inflammatory responses [34]. Furthermore, research into the mechanisms of action and clinical applications of traditional Chinese herbal formulas has been expanding in recent years, demonstrating potential benefits in modulating the intestinal microenvironment and alleviating UC symptoms [35]. Studies have demonstrated that Traditional Chinese Medicine possesses anti-inflammatory properties, which can lower the levels of inflammatory mediators such as IL-1 β and TNF- α . Additionally, it has been shown to reduce inflammatory lesions associated with UC in animal models [36, 37]. Furthermore, some research indicates that an imbalance in intestinal flora is closely linked to the onset and progression of inflammatory bowel diseases. Traditional Chinese Medicine may exert a therapeutic effect by influencing intestinal microecology and restoring the balance of intestinal flora [38, 39]. The therapeutic efficacy of QJN in treating UC can be largely attributed to its unique composition of multiple herbal ingredients, each contributing distinct biological activities that collectively enhance the formula's overall effectiveness. QJN includes a combination of herbs such as *A. membranaceus*, *C. chinensis*, and *P. ginseng*, which have been extensively studied for their individual and synergistic roles in managing inflammatory diseases, including UC. *A. membranaceus* is recognized for its immunomodulatory and anti-inflammatory properties [6]. Studies have demonstrated that its active compounds, such as astragalosides and polysaccharides, can effectively reduce inflammatory cytokines like IL-1 β and TNF- α , which are commonly elevated in UC. Additionally, these compounds enhance intestinal barrier function, preventing the translocation of harmful bacteria and toxins, thereby reducing inflammation and promoting mucosal healing [8, 9]. *C. chinensis*, which contains berberine, has been recognized for its ability to modulate gut microbiota and exert anti-inflammatory effects. Berberine inhibits the production of proinflammatory mediators and promotes the expression of tight junction proteins, thereby reinforcing the intestinal barrier. This mechanism aids in managing UC by both mitigating inflammation and restoring gut integrity [10, 11]. *P. ginseng* contains ginsenosides, which possess potent anti-inflammatory and antioxidative properties. The effectiveness of QJN is

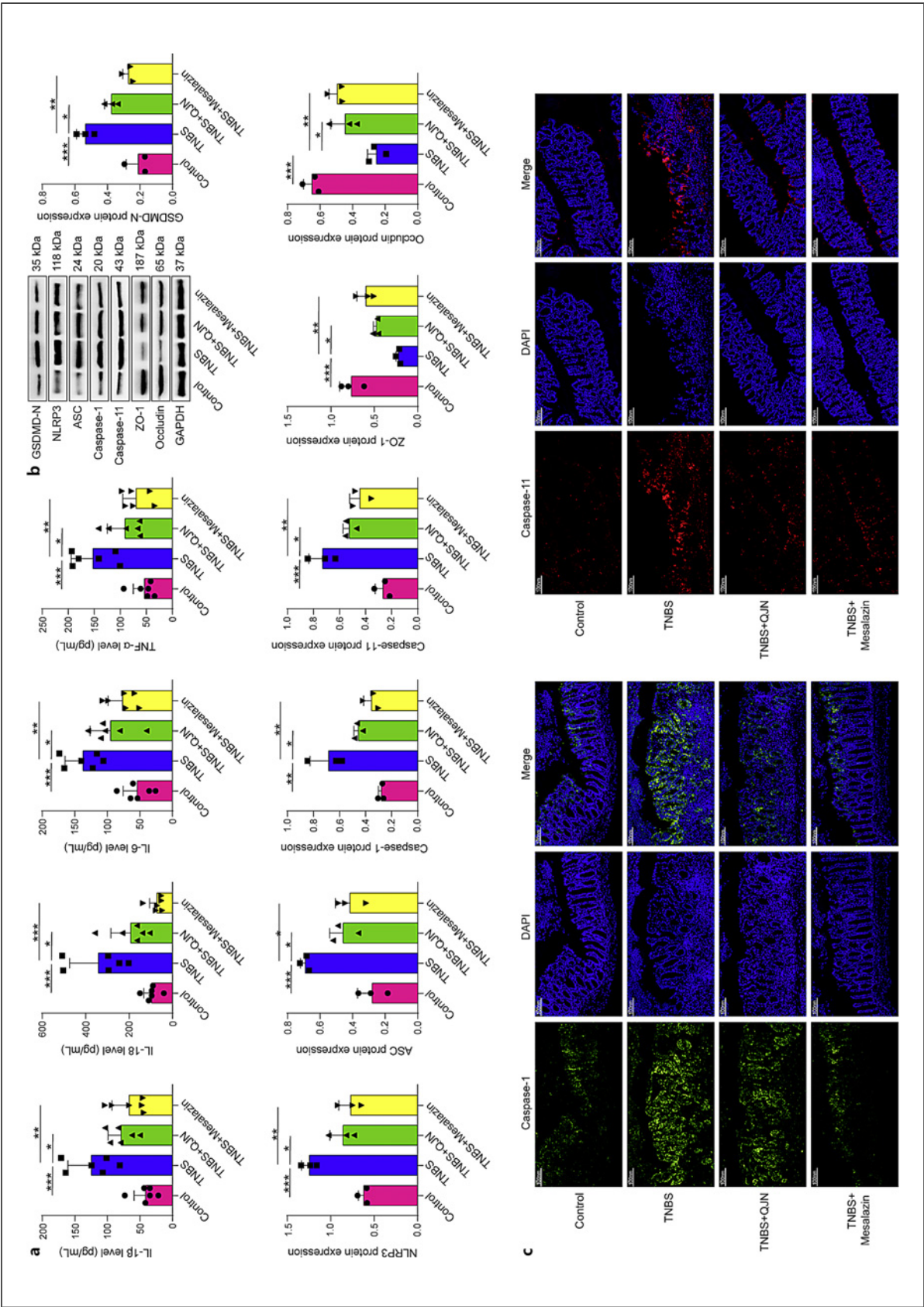


Fig. 6. QJN alleviates UC by suppressing the inflammasome-mediated inflammation via Caspase-1 and Caspase-11. **a** ELISA detection of serum levels of IL-1 β , IL-18, IL-6, and TNF- α . **b** Western blot analysis of GSDMD-N, NLRP3, ASC, activated Caspase-1, ZO-1, and Occludin. The presented blots were derived from the same gels. **c** Immunofluorescence detection of colonic levels of Caspase-1 and Caspase-11. The mice were divided into four groups: control, TNBS, TNBS plus QJN, and TNBS plus Mesalazine. Statistical differences were assessed using one-way ANOVA. TNBS group vs. control group, TNBS plus QJN group, and TNBS plus Mesalazine group vs. TNBS group. $n = 6$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

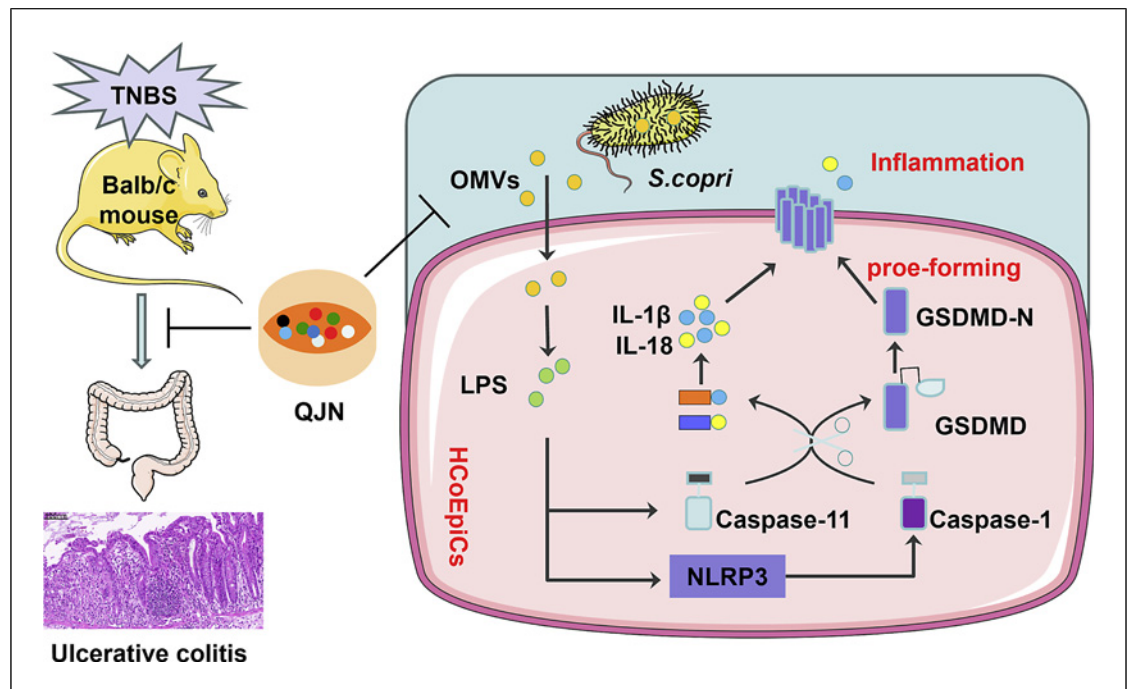


Fig. 7. Proposed mechanism of QJN in inhibiting inflammation in colonic epithelial cells and UC models. QJN treatment inhibits the production of OMVs from *S. copri* and reduces the LPS content in *S. copri*. The release of LPS activates the Caspase-11 and NLRP3/Caspase-1 pathways, leading to the conversion of pro-IL-1 β and pro-IL-18 into their active forms, IL-1 β and IL-18. In addition, activated Caspase-1 processes GSDMD into

GSDMD-N, which forms pores in cell membranes. The release of IL-1 β and IL-18 from these membranous pores triggers inflammatory responses. Furthermore, QJN inhibits the activation of these pathways, thereby reducing the production of inflammatory cytokines in colonic epithelial cells and mitigating the inflammatory injury of colons in TNBS-induced UC models.

not solely attributed to the individual actions of its components but also to their synergistic interactions [12]. The results of this study support the potential role of QJN and provide new insights into the complex interactions between gut microbiota and host cells, as well as the inflammatory signaling pathways.

OMVs have emerged as a focal point in the study of UC. Research has demonstrated that OMVs released by gut microbiota can transport a variety of bioactive substances, including LPS, proteins, and DNA fragments. These substances can directly interact with intestinal epithelial cells and the host immune system, triggering inflammatory responses [40, 41]. LPS and other inflammatory factors released by OMVs may compromise the integrity of the intestinal mucosal barrier and exacerbate intestinal inflammation [42–44]. In addition, OMVs can influence the intestinal immune response, disrupt the host's immune balance, and potentially accelerate disease progression [42]. Previous research has demonstrated that OMVs induce proinflammatory activation in macrophages through multiple Toll-like receptors [45, 46]. When inflammatory

responses are triggered in colonic epithelial cells, normal intestinal barrier function becomes compromised, leading to dysregulation of tight junction protein expression [47]. The current literature has well-characterized LPS-driven TLR4 signaling as a key player in the inflammatory cascade associated with UC [48]. However, previous studies have often concentrated on single-agent interventions targeting this pathway. Our study offers new insights by demonstrating that a complex herbal formula, such as QJN, can exert a modulatory effect on *S. copri* OMVs-induced HCoEpiC inflammation. This suggests that the interactions among its diverse constituents may have a synergistic effect in suppressing inflammation.

Caspase-1 and Caspase-11 are crucial effector proteins within the inflammasome complex, activated in response to various stress stimuli in cells, such as bacterial infections or signals of cellular damage. This activation leads to the cleavage and activation of precursor cytokines, including IL-1 β and IL-18, which in turn trigger inflammation [49]. In autoimmune diseases like UC, overactivation of Caspase-1 and Caspase-11 is linked to

pathological conditions [16]. This study demonstrated that QJN treatment significantly reduced serum levels of IL-1 β , IL-18, IL-6, and TNF- α in UC mice or *S. copri* OMVs-induced cells. This suggests that inhibiting the activity of Caspase-1 and Caspase-11 can decrease the production of key proinflammatory factors. The inflammasome, particularly the NLRP3 inflammasome, is a cytosolic complex that plays a key role in inflammation and cell death processes [29, 50–52]. This pathway involves the activation of various inflammatory mediators, including Caspase-1, an enzyme that processes proinflammatory cytokines such as IL-1 β and IL-18, converting them into active forms that initiate an inflammatory response. Studies have demonstrated that the inflammatory response can be effectively managed by inhibiting the activation of the NLRP3 inflammasome. This approach may aid in the treatment of inflammation associated with metabolic diseases, including UC [53].

Studies have shown that the combination of Mesalazine with traditional Chinese medicine may be more effective in treating UC [54–56]. This combined therapy demonstrates synergistic effects in reducing inflammation and improving intestinal mucosal healing. For instance, certain components of traditional Chinese medicine can enhance the anti-inflammatory effects of Mesalazine and jointly reduce the release of inflammatory mediators through various mechanisms. Additionally, traditional Chinese medicine can regulate gut microbiota and promote the restoration of intestinal barrier function. Based on these findings, it can be hypothesized that the combined use of QJN and Mesalazine may be more effective than using either treatment alone. QJN contains multiple herbal components with anti-inflammatory, immunomodulatory, and gut barrier-protective properties, which can enhance the anti-inflammatory mechanism of Mesalazine and create a synergistic effect. However, the specific efficacy of QJN in combination with Mesalazine has not yet been directly validated in current studies, which represents a limitation of the research. Future studies should design systematic experiments and clinical trials to evaluate the actual efficacy and safety of this combined therapy and further investigate the mechanisms underlying its synergistic effects. Such research will help clarify the potential benefits of combining QJN with Mesalazine, providing new scientific evidence for optimizing UC treatment strategies.

While our study focused on OMVs as mediators of *S. copri*-induced inflammation, we acknowledge that other interaction mechanisms between bacteria and host cells may also contribute to UC pathogenesis. Future studies should investigate whether QJN alters direct bacterial interactions with epithelial cells, influences the type and

immunostimulatory properties of LPS produced, or modifies bacterial protein expression profiles. Additionally, a proteomic analysis of OMVs from both QJN-treated and untreated *S. copri* would provide deeper insights into the specific molecular changes that contribute to a reduced inflammatory potential.

Conclusions

In conclusion (Fig. 7), QJN has been shown to inhibit the effects of OMVs released by *S. copri*, which carry LPS that trigger inflammatory responses. The presence of LPS activates Caspase-11 or the NLRP3 inflammasome, leading to the activation of Caspase-1. Activated Caspase-1 and Caspase-11 convert pro-IL-1 β and pro-IL-18 into their active forms, IL-1 β and IL-18, thereby promoting inflammation. Additionally, Caspase-1 processes GSDMD into its N-terminal fragment (GSDMD-N), which forms pores in cell membranes, further contributing to inflammation. QJN inhibits the activation of these pathways, reducing the production of inflammatory cytokines and the inflammatory responses in colonic epithelial cells and UC models. This study highlights the potential application of QJN in the clinical treatment of UC, providing promising outcomes for future clinical trials to verify the efficacy and safety of QJN.

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Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee of The Affiliated Hospital of Hunan Academy of Traditional Chinese Medicine (Approval No. SBWJW2024-0008).

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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research. Junjie Niu and Jinyang Hu performed experiment, collected the data, and analyzed the data. All authors reviewed the manuscript.

Author Contributions

Jinyang Hu guaranteed the integrity of the entire study, and designed the study. Shisheng Jiang and Yuhua Wu defined the intellectual content. Junjie Niu designed the study and literature

Data Availability Statement

The data that support the findings of this study are not publicly available due to ethics restrictions but are available from the corresponding author (Y.W.) (email address: 573631005@qq.com) upon reasonable request.

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