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Dexmedetomidine alleviates intestinal barrier dysfunction and inflammatory response in mice *via* suppressing TLR4/MyD88/NF-κB signaling in an experimental model of ulcerative colitis

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Abstract

Introduction. Ulcerative colitis (UC) is a nonspecific intestinal inflammatory disease. Dexmedetomidine (DEX) is a selective alpha 2-adrenergic receptor agonist commonly used for analgesia and sedation in intensive care units. Herein, the role and mechanism of DEX in dextran sulfate sodium (DSS)-induced colitis was explored.

Materials and methods. A murine model of DSS-induced colitis was established by adding 3.5% (w/v) DSS in drinking water to C57BL/6J female mice. The severity of colitis was measured by the disease activity index (DAI) score, colon length and body weight of mice. The serum concentration and mRNA levels of inflammatory cytokines in colon tissues were assessed by ELISA and RT-qPCR, respectively. Protein levels of apoptotic markers, tight junction proteins and genes involved in the TLR4/MyD88/NF-κB signaling were quantified utilizing Western blotting. The pathological changes of colon tissues were evaluated by hematoxylin-eosin (HE) staining and histological score. Intestinal permeability *in vivo* was assessed by fluorescein isothiocyanate (FITC)–dextran (FITC-D) administration. TUNEL assay was used to determine cell apoptosis in the intestinal epithelium.

Results. DSS administration resulted in weight loss, shortening of the colon, increased DAI score, histological abnormalities, and increased serum FITC-D levels in mice, all of which were reversed by DEX injection. Moreover, DEX attenuated DSS-triggered inflammatory response, intestinal barrier injury and intestinal epithelial cell apoptosis. Mechanically, DEX inactivated the TLR4/MyD88/NF-κB signaling in the colon tissues.

Conclusions: DEX exerts beneficial effects against the intestinal barrier dysfunction, inflammatory response, and apoptosis of intestinal epithelial cells *via* inactivation of the TLR4/MyD88/NF-κB signaling in mice with DSS-induced colitis. *(Folia Histochemica et Cytobiologica 2022, Vol. 60, No. 4, 311–322)*

Keywords: mouse; DSS; experimental colitis; dexmedetomidine; cytokines; TLR4/MyD88/NF-κB; intestinal barrier

Introduction

Inflammatory bowel disease (IBD) is a recurrent, idiopathic and chronic intestinal inflammatory disease [1]. According to an epidemiological study, IBD incidence in China reached 3.44%, ranking first in Asia [2]. Ulcerative colitis (UC) is a type of IBD and its clinical manifestation includes weight loss, bloody stools, abdominal pain, and diarrhea [3]. Due to its recurrent symptoms and poor prognosis, UC possibly develops into colitis-associated cancer, which has become an important public health problem in the world [4]. UC is caused by various factors such as unhealthy lifestyle, genetic susceptibility, microbial dysbiosis, and abnormal immune responses [5]. At present, the first-line drugs to treat UC mainly include biological agents, immunosuppressive drugs, aminosalicylates, and corticosteroids. However, high price or inevitable side effects of these drugs and the resistance to them

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hinder their clinical application [6]. Therefore, it is urgent to develop non-toxic, effective, and targeted agents for UC treatment.

Mucosal barrier dysfunction is considered the main cause of UC [7]. The epithelial barrier consists of a monolayer of epithelial cells with intercellular tight junctions composed of claudin-1, occludin, and ZO-1 between adjacent cells that seal the paracellular space and regulate barrier permeability [8]. A large number of environmental antigens and external microorganisms can reach the epithelium or underlying lamina propria *via* the mucous membrane [9]. Consequently, the integrity of the intestinal epithelium is critical to the prevention of intestinal infection, which is associated with immune perception [9]. The defect or integrity of the epithelial barrier is an important pathogenic marker for inflammatory intestinal diseases [10]. Intestinal barrier dysfunction results in the enhancement of epithelial permeability and inflammation [11].

Dexmedetomidine (DEX) is an α2-adrenergic agonist that exerts sedative, analgesic, and hypotensive effects with less respiratory depression than other sedative drugs [12]. Systemic administration of DEX can reduce opioids' requirements by enhancing the analgesic effects of opioids during the perioperative period [13]. DEX can restrain inflammation in various types of tissue injury, such as neuropathic pain [14], acute kidney injury [15], and sepsis [16]. Accumulating studies have revealed the protective role of DEX against intestinal injury [17]. For example, DEX pretreatment significantly ameliorates intestinal pathological symptoms and intestinal inflammation in rats with intestinal ischemia/reperfusion injury [18] and protects against deprivation/reoxygenation-induced human colon carcinoma cell damage *in vitro via* suppressing the TLR4/MyD88/NF-κB signaling [18]. DEX combined with lidocaine infusion can remarkably enhance bowel function recovery and mitigate the postoperative pain of patients undergoing abdominal hysterectomy [19]. DEX protects against intestinal cell apoptosis *via* restricting cytochrome c release and mitochondrial depolarization mediated by p38 MAPK and inhibits ischemia/reperfusion-induced intestinal inflammation *via* suppressing p38 MAPK activity [20]. Notably, DEX was reported to mitigate 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice by inducing an anti-inflammatory effect [21]. Specifically, DEX increases IL-4 and IL-10 (anti-inflammatory factors) levels in serum of model mice *via* Th2 pathway while reducing IL-23 (inflammatory factor) level *via* Th17 pathway [21]. However, whether and how DEX mitigates UC pathogenesis remains unknown. Based on the findings from pre-

vious studies, it can be hypothesized that DEX might regulate intestinal barrier functions and inflammatory response to alleviate intestinal injury *in vivo*.

To verify the hypothesis, a murine model of dextran sulfate sodium (DSS)-induced colitis was established. Afterward, the pharmacological function and specific mechanism of DEX in the model were investigated. Our study provides a better understanding of the biological and pharmacological properties of DEX and reveals the potential role of DEX in alleviating DSS-induced colitis.

Materials and methods

Animal experiments. A total of 40 female C57BL/6J mice (22––25 g, 6-week-old), were purchased from SPF Animals Biotechnology (Beijing, China). Mice were raised in a room with a 12 h light/12 h dark cycle at 25°C and with *ad libitum* access to food and water. Mouse diet composition is listed in Suppl. Table 1. For the establishment of an animal model, mice were randomly divided into four groups: sham, sham + DEX, DSS, and DSS + DEX (n = 10 *per* group). As previously described [22], mice in the sham + DEX and DSS + DEX groups were intraperitoneally injected with 30 μg/kg DEX (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) dissolved in phosphate-buffered saline (PBS: 30 mM KCl, 15 Mm Na2HPO4, 150 mM NaCl, 2 mM KH2PO4, pH7.4) once a day for 14 days. Mice in the sham and DSS groups received the same volume of PBS once a day for 14 days by *i.p.* injection. The dose of DEX used in this study was based on the data from the previous study [21]. From day 7 to day 14, mice in the DSS and DSS+DEX groups received 3.5% (w/v) DSS in drinking water. Mice in the sham and sham + DEX groups had *ad libitum* access to untreated drinking water from day 1 to day 14. Body weight and disease activity index (DAI) score in each group was calculated from day 7 to day 14. DAI was assessed in combination with body weight loss, mucous stool, and hematochezia scores [23]. The scoring criteria for DAI are detailed in Supplementary Table 2. On day 15, mice were fasted for 18 h and then anesthetized through *i.p.* injection of pentobarbital sodium (30 mg/kg). Afterward, blood samples were collected from mice using cardiac puncture. At termination, mice were euthanized by cervical dislocation. Then, colon samples were collected, and colon lumen content was washed with PBS. The obtained colon tissues were frozen in liquid nitrogen and then stored at −70°C until further study. The colon tissues were fixed in 4% paraformaldehyde for 24 h, dehydrated in ethanol gradients (30%, 50%, 70%, 80%, 90%, 95%, 30 min each), and rendered transparent through immersion in xylene. Finally, the tissues were embedded in paraffin.

This study was approved by the Ethics Committee of Jinshan Hospital of Fudan University, China, and all the protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals provided by the National Institutes of Health, USA.

Table 1. Primer sequences used for real-time PCR analysis of gene expression

Gene	Primer	Oligonucleotide sequence (5′-3′)
IL-1β	Forward	CTTTCCCGTGGACCTTCCAG
	Reverse	AATGGGAACGTCACACACCA
IL-6	Forward	TGCCTTCTTGGGACTGATGC
	Reverse	TGAAGTCTCCTCTCCGGACT
TNF-α	Forward	CTCATGCACCACCATCAAGG
	Reverse	ACCTGACCACTCTCCCTTTG
GAPDH	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	GGGGTCGTTGATGGCAACA

Hematoxylin-eosin (HE) staining of intestinal tissues. The paraffin-embedded colon tissue was cut into 4 μm-thick serial sections, deparaffinized with xylene, dehydrated with gradient ethanol, stained with hematoxylin for 3 min, supplemented with 1% hydrochloric acid alcohol for 2 s, incubated with 1% ammonia water for 20 s, treated with 0.5% eosin alcohol for 10 s, dehydrated with gradient alcohol, cleared by xylene and mounted using neutral gum. The histopathological changes of intestinal tissues were observed under a light microscope (Olympus, Tokyo, Japan). Histological characteristics of colon tissue specimens were scored independently as previously described [24]. Detailed criteria for histology are shown in Supplementary Table 3.

Enzyme-linked immunosorbent assay (ELISA). For ELISA, serum was extracted from clotted blood after centrifugation at 850 g for 20 min at 4°C. Interleukin 1 beta (IL-1β), tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) were evaluated using commercial ELISA kits (Neobioscience Technology Co., Ltd., Shenzhen, China) based on the manufacturer's guidelines.

RT-qPCR. TRIzol (TianGen, BeiJing, China) was utilized for extracting total RNA from colon tissues. The quality and concentration of RNA were quantified by the NanoDrop 2000C spectrophotometer (ThermoFischer Scientific, Waltham, MA, USA). Total RNA was reverse transcribed using cDNA Prime Script RT Master Mix (TaKaRa, Beijing, China). Transcription levels of specific genes were detected by RT-qPCR analysis using the TB Green Premix Ex Taq II (TaKaRa, Beijing, China) on Light Cycler96 system (Roche, Mannheim, Germany). *GAPDH* was set as an internal control. Primer sequences are shown in Table 1. Relative gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method.

Measurement of intestinal permeability. Intestinal permeability was assessed *via* the fluorescein isothiocyanate (FITC)-labelled dextran method as described by Volynets *et al.* [23]. In brief, on the last day of the experiment, mice orally received 600 mg/kg FITC-dextran (MW 3000–5000 kDa, Sigma-Aldrich, St. Louis, MO, USA) 4 h before euthanasia. Then, blood was collected from mice and heparinized just before euthanasia. The plasma was centrifuged at 12000 g at 4° for 10 min, and 96-well black microplates were supplemented with 200 μL of

each sample. Fluorescence was read at 485/528 nm using Agilent Biotek Synergy H4 (Santa Clara, CA, USA).

Measurement of intestinal epithelial cell apoptosis. The terminal deoxynucleotidyl transferase-mediated deoxyuridine-triphosphate nick-end labeling (TUNEL) assay was utilized for detecting apoptotic cells in the intestinal epithelium. According to the manufacturer's protocol, DeadEnd TM Fluorometric TUNEL system (Promega, Madison, WI, USA) was used to label deparaffinized and rehydrated colon tissue sections [25]. As previously described, apoptosis was quantified by evaluating TUNEL-positive crypts in 80 to 100 randomly selected crypts in 10 mice per group, and the apoptotic index referred to the percentage of TUNEL-positive cell-containing crypts [26].

Western blotting. After colon tissues collected from mice were cut into small pieces, RIPA lysis buffer (Beyotime, Nanjing, China) containing protease inhibitors was used to treat the tissue samples. A BCA assay kit (Beyotime) was used to quantify the obtained proteins. Based on SDS-PAGE electrophoresis, proteins were transferred onto PVDF membranes. The membranes were blocked with 5% skimmed milk powder for 2 h, followed by incubation with primary antibodies (all from Abcam, Cambridge, MA, USA) at 4°C overnight. Subsequently, HRP-labelled goat anti-rabbit secondary antibody (Abcam, 1:2000) was incubated with the membranes at room temperature for 2 h. After incubation, the membranes were visualized with an enhanced chemiluminescence (ECL) kit (Shanghai Sangon, China), followed by optical density analysis by Image-Pro Plus 6.0 software (Medium Cybernetics, Bethesda, MD, USA). Primary antibodies used for Western blotting (all from Abcam) were as follows: anti-Claudin-1 (ab211737, 1:2000), anti-Occludin (ab216327, 1:1000), anti-ZO-1 (ab216880, 1:1000), anti-Bcl-2 (ab182858, 1:2000), anti-Bax (ab32503, 1:1000), anti-Cleaved caspase-3 (ab214430, 1:5000), anti-MyD88 (ab219413, 1:1000), anti-TLR4 (ab13556, 1:500), anti-p65 (ab32536, 1:1000), anti-p-p65 (ab76302, 1:1000), anti-IκBα (ab32518, 1:1000), anti-p-IκBα (ab92700, 1:1000) and GAPDH (ab9485, 1:2500). GAPDH was set as a loading control.

Statistical analysis. The data obtained from at least three experiments were presented as the mean ± standard deviation and statistics were analyzed by GraphPad Prism software 6.0 (GraphPad Inc., San Diego, CA, USA). Student's *t*-test and one-way analysis of variance (ANOVA) followed by Tukey *post hoc* analysis were utilized for comparison between two groups and in multiple groups, respectively. The value of $P < 0.05$ was considered statistically significant.

Results

DEX attenuates DSS-induced colitis

DSS was used to induce colitis in mice, and the role of DEX in the murine model of DSS-induced colitis was explored. The experimental schedule was illustrated in Fig. 1A. As expected, the DSS group exhibited on day 14 shortening of the colon (40% decrease, Fig. 1B) and
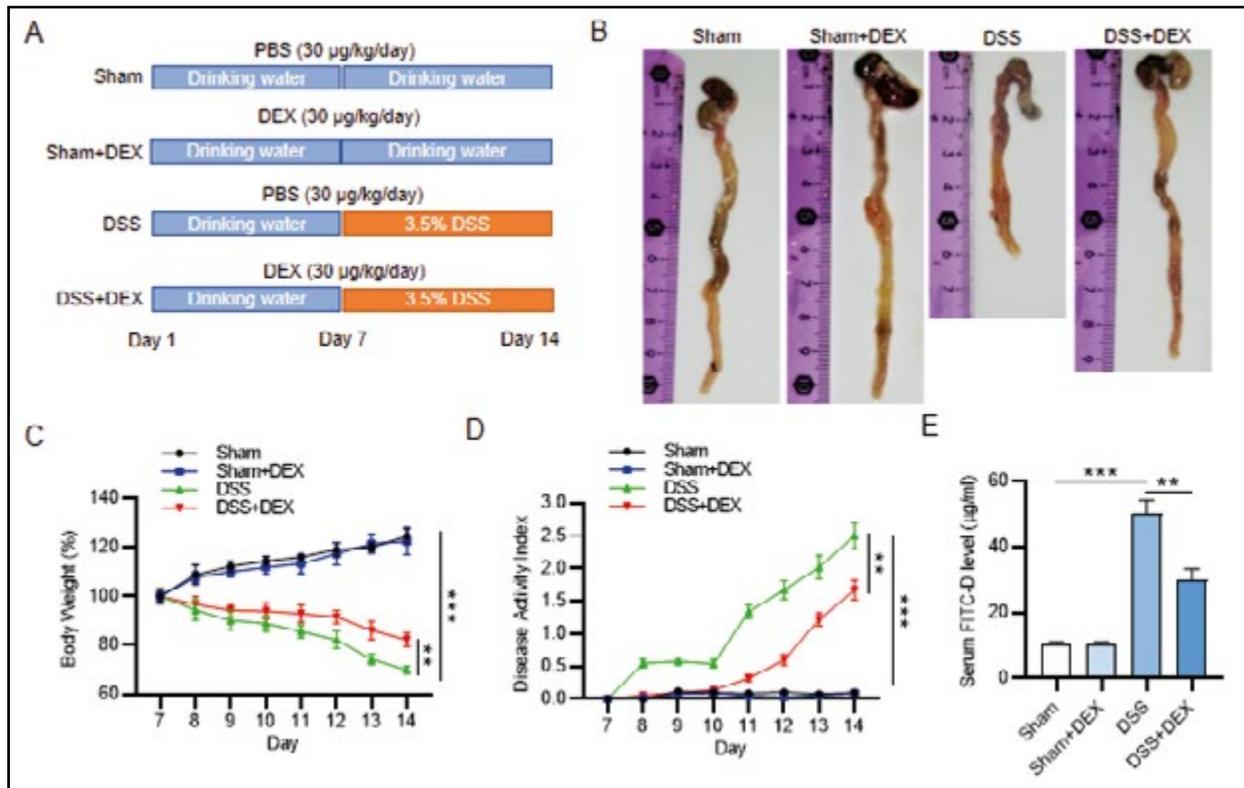
Figure 1. Dexmedetomidine (DEX) attenuates dextran sulfate sodium (DSS)-induced colitis in female mice. **A.** Experimental design to explore the impact of DEX on DSS-induced colitis (sham group, sham + DEX group, DSS group, and DSS + DEX group). **B.** Representative images of colons from mice in each group and the length of these colons. **C.** Body weight of mice in each group during the course of colitis (from day 7 to day 14). **D.** Disease activity index (DAI, criteria listed in Suppl. Table 2) score of mice from day 7 to day 14 in each group. **E.** Serum Fluorescein isothiocyanate-dextran (FITC-D) levels on day 14. Experiments were performed as described in Material and methods. N = 10 mice/group. ** $P < 0.01$, ***$P < 0.001$.

weight loss (43.8% decrease, Fig. 1C) compared to the sham-operated group. However, the decrease in colon length and body weight induced by DSS administration was partially reversed by DEX injection (46% increase in colon length; 17.6% increase in body weight on day 14) (Fig. 1B, C).

Moreover, the DSS group displayed increased DAI score relative to the sham group, and the score in the DSS+DEX group was decreased compared with that in the DSS group (Fig. 1D). Data for mean colon length, body weight and DAI score in four experimental groups are available in Supplementary Tables 1–3.

Intestinal permeability was assessed by measurement of FITC-labelled dextran levels in serum. The outcome demonstrated that the DSS group had markedly higher serum FITC-D levels than the sham group, while DEX administration neutralized DSS-induced increase in FITC-D levels (Fig. 1E), suggesting that DEX administration for 14 days alleviates DSS-enhanced intestinal permeability.

DEX alleviates DSS-induced histological abnormalities of colon tissues

Based on the results of the HE staining and the histological score, DSS treatment contributed to histological abnormalities of colon tissues. As shown in Fig. 2A, the mucosa and muscular layer of the colon tissue was inflamed in DSS group. In addition, the mucosal layer in DSS group was ulcerated together with the disappearance of many goblet cells. The colon gland was also disappeared and was replaced by infiltrating inflammatory cells in the DSS group. The above histopathological changes were attenuated by DEX administration. Mice treated with DEX had decreased histopathologic score compared to those in DSS group (Fig. 2B). The characteristic features of injury were marked with black arrows. Moreover, images of colon sites expressing selected tissue fragments with inflammatory features are provided in Supplementary Fig. 1. The above findings indicated that DEX alleviates the clinicopathological characteristics of DSS-induced colitis in mice and mitigates colon injury.
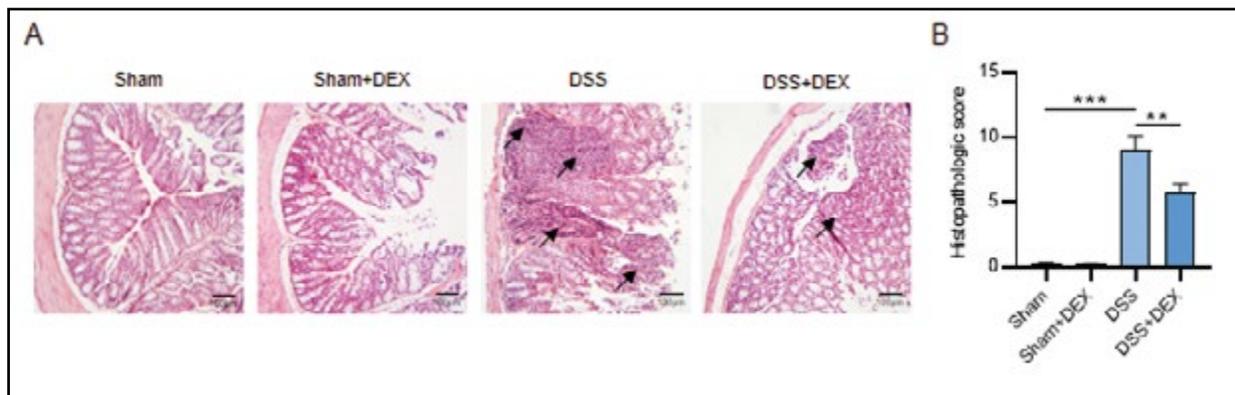
Figure 2. DEX alleviates DSS-induced histological abnormalities of colon tissues. **A.** Representative image of HE-stained colon tissue samples in each group; scale bar: 100 μm. **B.** Histological score of colonic tissues in each group based on HE staining and criteria listed in Suppl. Table 3. ** P < 0.01, ***P < 0.001.
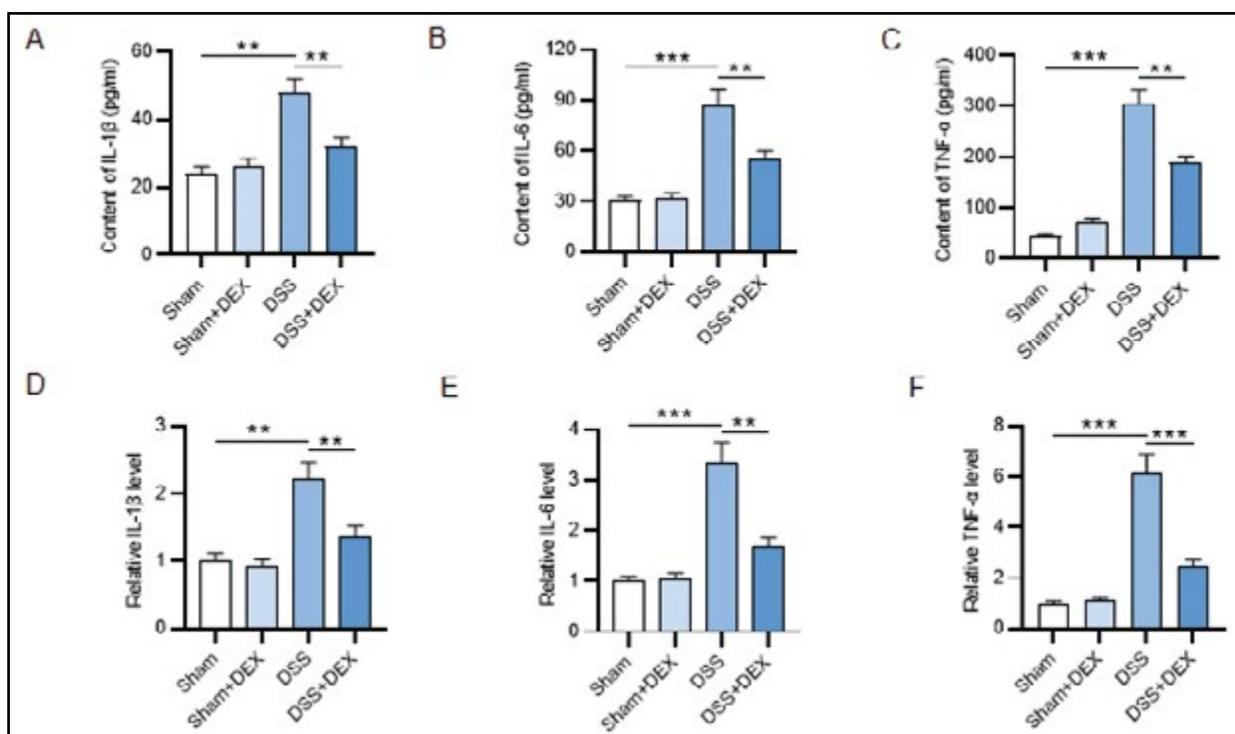

Figure 3. DEX attenuates DSS-triggered inflammatory response. **A–C.** ELISA was used to evaluate the concentration of proinflammatory cytokines (IL-1β, TNF-α, IL-6) in sera of mice in sham, sham + DEX, DSS, and DSS + DEX groups. **D–F.** RT-qPCR to determine relative mRNA expression of the inflammatory factors in murine colon tissue. N = 10 mice/group. ** P < 0.01, ***P < 0.001.

DEX reduces levels of inflammatory cytokines in mice

ELISA was performed to evaluate the serum levels of proinflammatory cytokines (IL-1β, TNF-α, IL-6). Compared with the sham group, the administration of DSS markedly elevated serum IL-1β, IL-6 and TNF-α levels in mice (Fig. 3A–C). Moreover, this tendency was remarkably reversed by DEX administration (Fig. 3A–C).

The results of RT-qPCR analysis showed that DSS treatment significantly increased mRNA levels of the proinflammatory cytokines in murine colon tissues, and the alternations were countervailed by DEX administration (Figs. 3D–F). In summary, DEX alleviates DSS-induced colonic inflammation of colon mucosa in the murine model.

DEX prevents intestinal barrier damage in mice

To assess the effects of DEX administration on intestinal barrier integrity, the mRNA and protein levels of tight junction proteins (claudin-1, occludin, and ZO-1) were measured using RT-qPCR and Western blotting,
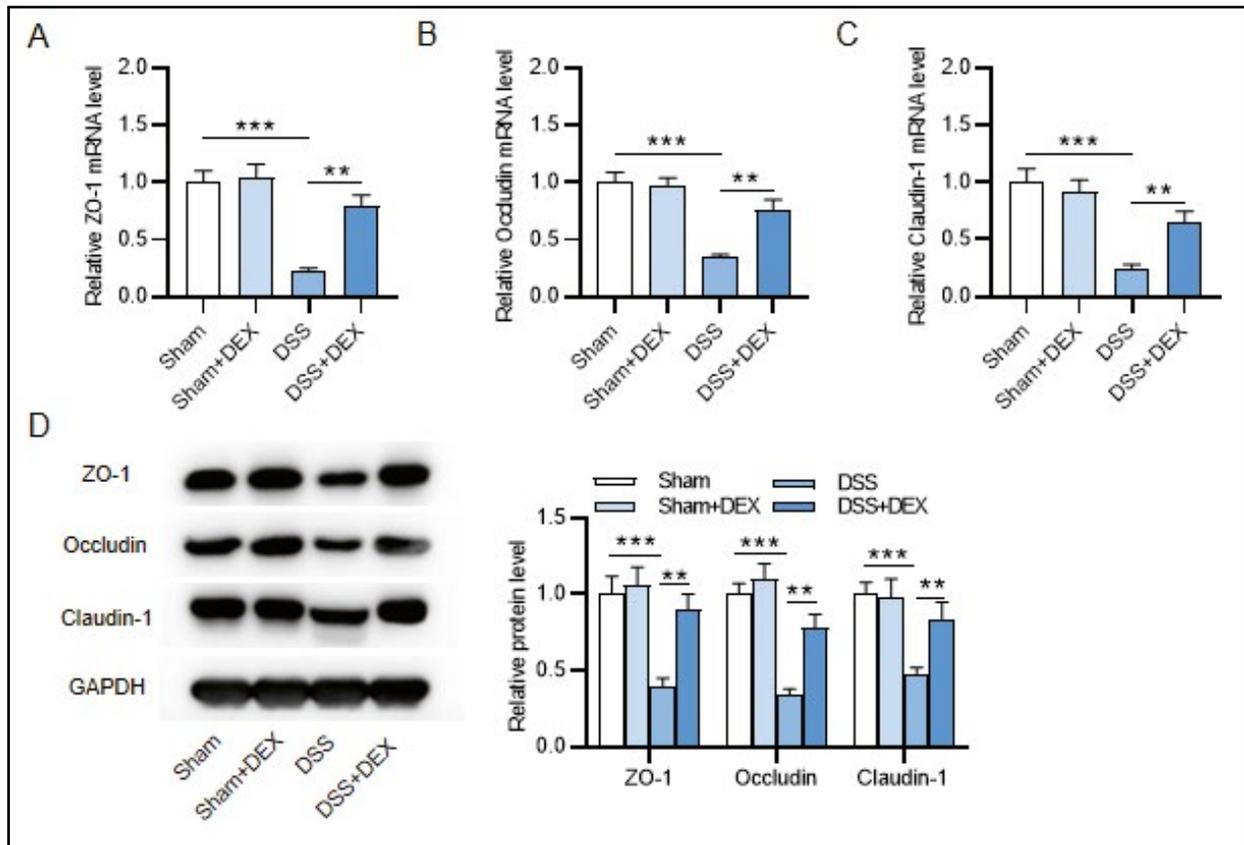
Figure 4. DEX prevents intestinal barrier damage in mice. **A–C.** RT-qPCR was used to analyze mRNA expression of ZO-1, occludin, and claudin-1 in murine colon tissues of mice from sham, sham + DEX, DSS, and DSS+DEX groups. **D.** Protein levels of ZO-1, occludin, and claudin-1 in murine colon tissues were quantified by Western blotting. N = 10 mice/group. $^{**}P < 0.01$, $^{***}P < 0.001$.

respectively. It was found that mice pretreated with DSS exhibited significantly lower mRNA and protein levels of claudin-1, occludin, and ZO-1 compared with mice in the sham group (Fig. 4A–D). However, the decrease in the expression of the tight junction proteins at the mRNA and protein levels triggered by DSS administration was rescued by DEX pretreatment (Fig. 4A–D). Taken together, DEX exerts a protective effect against intestinal barrier disruption in the murine model of DSS-induced colitis.

DEX suppresses DSS-induced apoptosis of intestinal epithelial cells

It is known that intestinal epithelial cell apoptosis leads to the dysfunction of the intestinal barrier [27]. Next, TUNEL staining and Western blotting were used to test the impact of DEX on the apoptotic index of intestinal epithelium in the studied experimental model. As Fig. 5A demonstrated, the number of apoptotic cells in the intestinal epithelium of the DSS group was more than that in the sham group, and DEX pretreatment alleviated DSS-enhanced intestinal epithelial cell apoptosis. Moreover, Western blotting revealed that DSS administration contributed to increased cleaved caspase-3 and

Bax protein levels and decreased Bcl-2 protein levels in murine colon tissues, and these alternations were reversed by DEX administration (Fig. 5B). Thus, it may be concluded that DEX inhibits DSS-induced intestinal epithelial cell apoptosis.

DEX inactivates the TLR4/MyD88/NF-κB signaling in an *in vivo* ulcerative colitis model

External stimuli-induced intestinal innate immune responses contribute to severe intestinal mucosal epithelial abnormalities by upregulating TLR4 expression and activating NF-κB through MyD88 dependent signaling [28, 29]. Therefore, we decided to test whether DEX can suppress TLR4/MyD88/NF-κB signaling in DSS-induced colitis. Protein levels of genes involved in the pathway were quantified utilizing Western blotting. As Fig. 5A revealed, the DSS-induced mice exhibited an increase in TLR4, MyD88, p-p65, and p-IκBα protein levels compared with the sham group, suggesting that DSS administration activates the TLR4/MyD88/NF-κB signaling in colon tissues. However, DEX administration reversed the promoting effect of DSS treatment on TLR4, MyD88, p-p65, and p-IκBα protein levels in colon tissues
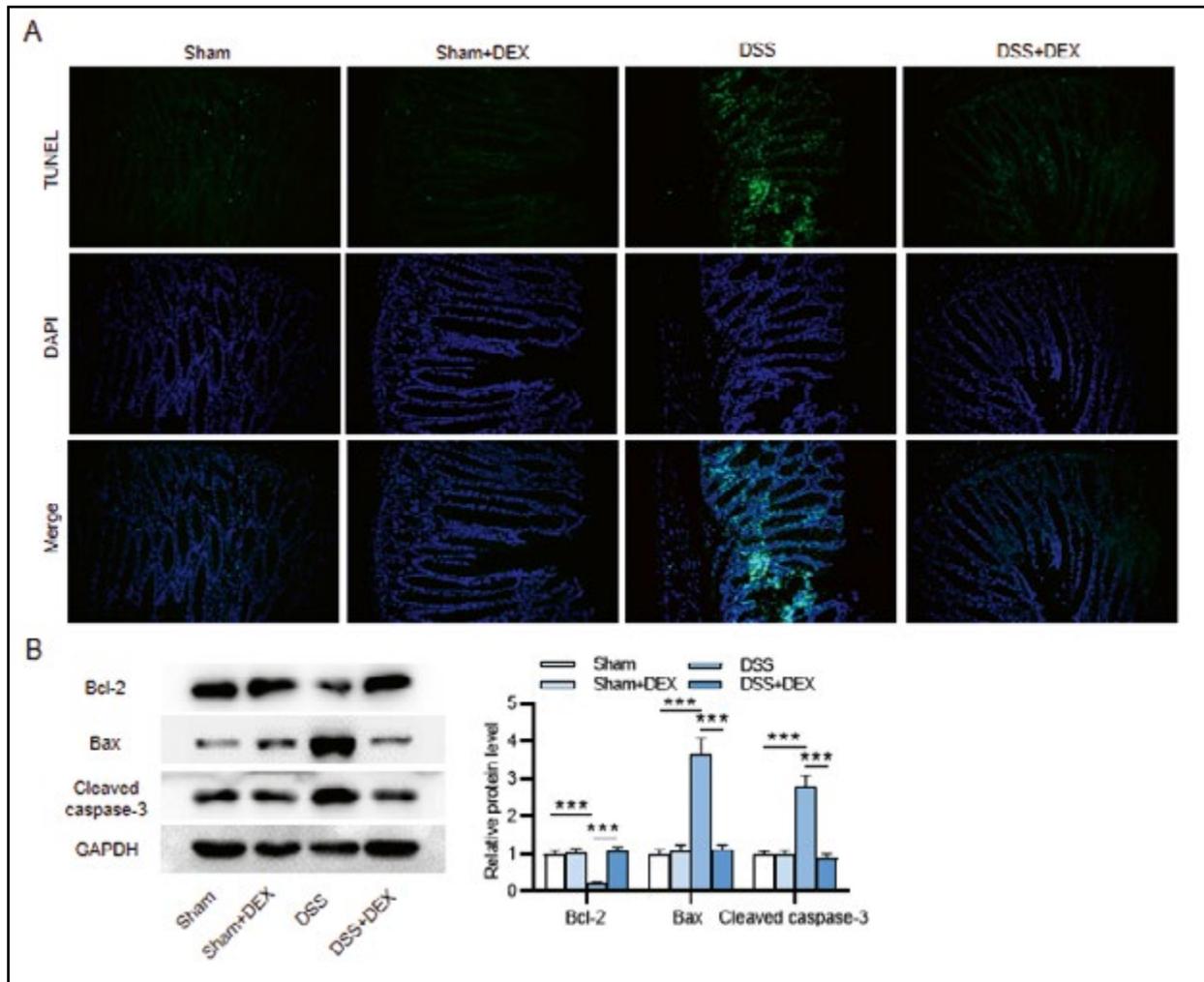
Figure 5. DEX suppresses DSS-triggered apoptosis of intestinal epithelial cells. **A.** TUNEL assay was used to evaluate intestinal epithelial cell apoptosis in mice from sham, sham + DEX, DSS, and DSS + DEX groups. **B.** Western blotting was used for quantifying protein levels of apoptosis markers (Bcl-2, Cleaved caspase-3 and Bax) in murine colon tissues. N = 10 mice/group. ***P < 0.001.

(Fig. 6A). To sum up, we demonstrated that DEX inhibits DSS-induced activation of TLR4/MyD88/NF-κB signaling *in vivo*.

Discussion

UC is an incurable and relapsing idiopathic type of IBD [30]. DSS has been widely used to establish experimental UC in mouse models [31]. The clinical phenotypes in animal models were highly similar to those in UC patients, including the increase in DAI [32]. Additionally, model mice show pathological features of colitis, such as mucosal barrier damage, immune cell infiltration, and intestinal micro-dysbiosis [33]. In this study, a murine model of DSS-induced colitis was established. As expected, mice with DSS-triggered colitis exhibited clinicopathological characteristics including the shortening of the colon, weight loss, histological abnormalities of colon tissues, and increased DAI score. In previous studies, intestinal permeability in mice with DSS administration has been assessed successfully *via* the FITC-dextran method [34]. Consistently, in our study, DSS-treated mice exhibited high serum FITC-dextran levels, indicating that DSS treatment increased intestinal permeability.

Pathological changes related to UC include ulcer formation, intestinal epithelial barrier disruption, and gut inflammation [35]. The intestinal epithelial barrier is made up of epithelial cell monolayer with intercellular tight junctions composed of claudin-1, occludin, and ZO-1 that modulate intestinal permeability [36–39]. Excessive apoptosis of intestinal epithelial cells leads to intestinal barrier dysfunction and has been considered as a main pathogenic mechanism of chronic intestinal inflammation [40]. Previously, 1 mL of 5% acetic acid was administered to male C57BL/6 mice to
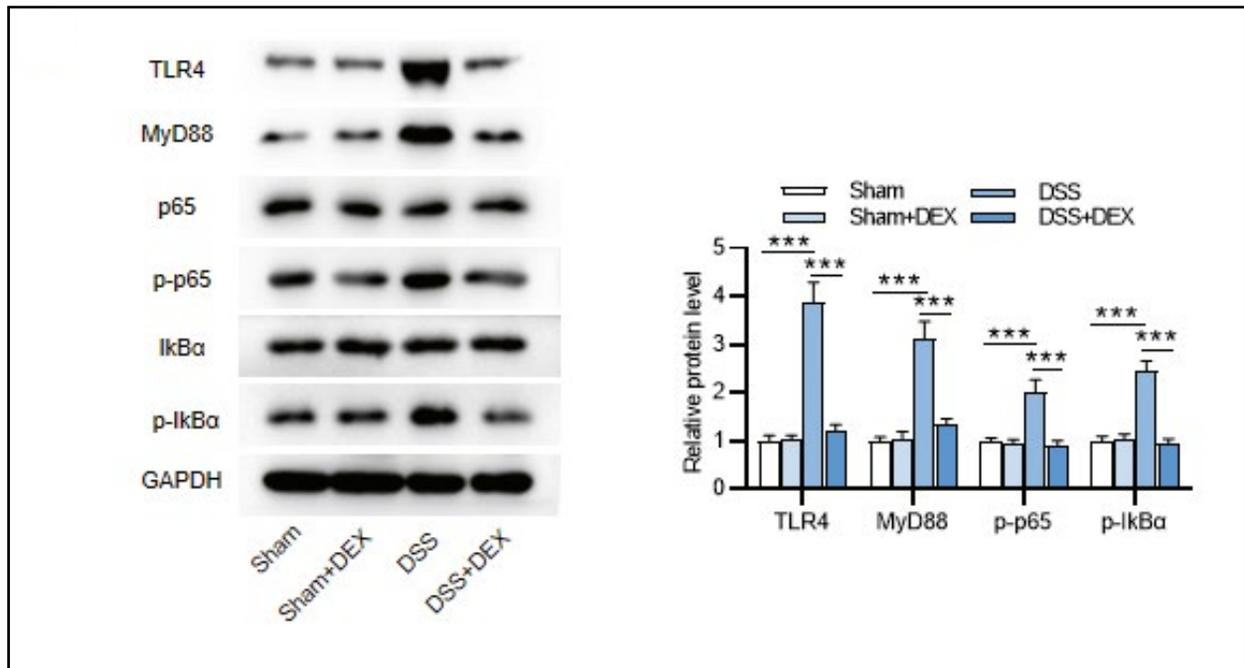
Figure 6. DEX inactivates the TLR4/MyD88/NF-κB signaling in colon tissues of mice with DSS-induced colitis. Western blotting to quantify protein levels of TLR4, MyD88, p-p65, p65, p-IκBα and IκBα in murine colon tissues of sham, sham + DEX, DSS, and DSS + DEX groups. N = 10 mice/group. ***P < 0.001.

induce UC; then, serum levels of inflammatory cytokines (IL-6 and TNF-α) were increased and colonic cell apoptosis was induced [41]. In male Kunming mice treated with 3% DSS gavage for 5 days, decreased tight junction protein levels (claudin-1, occludin, and ZO-1) in colon samples were found [42]. Moreover, UC can also be induced in male Balb/c mice treated with 5 mg of trinitrobenzenesulfonic acid (TNBS; dissolved in 0.2 mL of 50% ethanol); inflammation and oxidative stress were induced and protein levels of pro-apoptosis molecules (Bax, caspase-3, caspase-9, and cytochrome C) were increased in colon tissues [43]. These findings were consistent with the results of our study. Our results showed that 3.5% DSS treatment for 7 days in female C57BL/6J mice induced the increase in serum concentrations of inflammatory cytokines (IL-1β, IL-6, and TNF-α), demonstrating that DSS induced inflammatory response in mice. Moreover, DSS treatment contributed to the damage of intestinal barrier integrity, which was consistent with the previous findings in Sprague–Dawley rat model of UC [44] and Kunming mouse model of UC [42].

As a potent α2-adrenoceptor agonist 1, DEX is widely used to sedate patients on mechanical ventilation in intensive care units [45]. It was reported that DEX plays a protective role in gut surgery since the usage of DEX during the perioperative period markedly decreased the time of initial flatus, defecation, and intestinal recovery [46]. In endotoxemic rats, DEX attenuates tight junctional damage, mucosal cell death, and intestinal microcirculatory dysfunction, protecting against the disruption of the intestinal epithelial barrier [47]. Perioperative administration of DEX alleviates intestinal injury in patients with selective hepatectomy and inflow occlusion [48]. DEX usage before ischemia partly inhibits intestinal epithelial apoptosis and inflammatory response to protect against intestinal injury induced by ischemia/reperfusion in a dose-dependent manner [49]. DEX can mitigate intestinal permeability, inflammatory response and intestinal mucosal damage caused by traumatic brain injury, suggesting that DEX may ameliorate intestinal tissue injury *via* regulating inflammation after traumatic brain injury [50]. Our data revealed that DEX injection ameliorated DSS-induced pathological symptoms by reversing the effects of DSS on colon length, body weight, DAI score, and histopathologic score. Additionally, the elevation of FITC-dextran levels induced by DSS in mice was offset by DEX administration, suggesting that DEX pretreatment improves intestinal permeability induced by DSS exposure. The protective effect of DEX against intestinal epithelial barrier breakdown was observed in DSS-induced normal colonic epithelial cells (NCM460), as evidenced by an increase in levels of tight junction proteins (ZO-1, occludin, and claudin-1) compared with those in DSS-treated NCM460 cells [51]. DEX inhibited DSS-induced colitis which was also documented by decreased serum

concentration of inflammatory factors. Similarly, in the experimental model of TNBS-induced colitis, DEX exhibits an anti-inflammatory impact by increasing serum levels of anti-inflammatory factors (IL-4 and IL-10), which highlights the significant role of DEX in inhibiting inflammatory immune response in a tissue-specific manner [21]. A reduction of the serum levels of inflammatory factors mediated by DEX was also demonstrated in an *in vitro* cellular model of UC [51]. The administration of DEX to mice with DSS-induced colitis also maintained intestinal barrier integrity by increasing levels of tight junction proteins and suppressing intestinal epithelial cell apoptosis. Similar effects on epithelial cell apoptosis mediated by DEX were observed in DSS-induced *in vitro* cell model for UC [51]. Therefore, DEX exerts anti-inflammatory and anti-apoptotic effects in DSS-induced colitis.

According to a previous study, TLR4/MyD88/NF-κB signaling is associated with intestinal inflammation in UC [52, 53], and DEX was shown to inhibit TLR4/MyD88/NF-κB signaling in septic acute kidney injury [54]. Therefore, whether DEX can regulate this pathway in the experimental UC model was investigated in our study. In the innate immune system, toll-like receptors (TLRs) are one of the pattern recognition receptors and TLRs imbalance can exacerbate acute inflammatory mucosal damage [55]. Activated TLRs can transmit information about inflammatory response through MyD88 signaling and mediate proinflammatory cytokine secretion, subsequently causing tissue injury [56]. As an ubiquitous transcription factor, NF-κB plays an important role in gene regulation, inflammatory responses, immune modulation, and apoptosis [57]. When IκBα is phosphorylated and degraded, the occurrence of p65 phosphorylation and nuclear translocation activates NF-κB signaling to alter inflammatory gene expression [58]. Numerous reports demonstrated the involvement of NF-κB signaling in animal models of UC and mucosa of patients with UC. For example, the aqueous-methanol extract was shown to ameliorate DSS-triggered UC in mice *via* maintaining intestinal barrier function and inhibiting the inflammatory response by regulating NF-κB and MAPK pathways [59]. Butyrate suppresses the activation of NF-κB signaling in lamina propria macrophages of patients with UC, thus decreasing DAI score and mucosal inflammation in butyrate-treated patients [60]. Eriodictyol treatment alleviates TNBS-induced colon inflammation and intestinal tissue damage in rats by inactivating the TLR4/NF-κB signaling [61]. Indigo and indirubin repair intestinal mucosa, reduce inflammation, regulate intestinal flora, and ameliorate physiological symptoms of DSS-induced colitis in mice *via* repressing TLR4/MyD88/NF-κB signal

transduction [62]. In our study, DSS exposure elevated the levels of TLR4, MyD88, p-p65 and p-IκBα in colon tissues. However, all these effects induced by DSS were mitigated by DEX administration. Thus, DSS-caused activation of the TLR4/MyD88/NF-κB signaling was suppressed by DEX injection *in vivo*.

In conclusion, we found that the pretreatment of mice with DEX alleviates DSS-induced pathological changes, inflammatory response, intestinal barrier damage and intestinal epithelial cell apoptosis in an experimental mouse model of UC by inactivating the TLR4/MyD88/NF-κB pathway. Our study suggested that DEX may serve as a preventive therapeutic agent for UC treatment. However, more explorations about whether DEX can regulate other signaling pathways are needed in the future.

Conflict of interest

The authors declared no conflict of interest.

Ethics approval

This study was approved by the Ethics Committee of Jinshan Hospital of Fudan University and all the protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals provided by the National Institutes of Health.

Authors' contributions

All three authors participated in the literature search, analysis and interpretation of the data, and the writing of the manuscript. All authors saw and approved the final manuscript.

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