



## Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica,  
Alergología y Asma Pediátrica

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ORIGINAL ARTICLE

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# Dexmedetomidine reduces dextran sulfate sodium (DSS)-induced NCM460 cell inflammation and barrier damage by inhibiting RhoA/ROCK signaling pathway

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Received 24 December 2021; Accepted 15 February 2022

Available online 1 May 2022

### KEYWORDS

dexmedetomidine;  
inflammation;  
barrier damage;  
RhoA/ROCK signaling  
pathway;  
ulcerative colitis

### Abstract

**Objective:** This study investigated the role of dexmedetomidine (DEX) in dextran sulfate sodium (DSS)-induced NCM460 cells.

**Material and Methods:** The viability and apoptosis of NCM460 cells treated with DEX with or without DSS were detected by CCK-8 and terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay. The level of inflammatory factors and expression of inflammation-related proteins, tight junction proteins and Ras homolog gene family, member A/Rho-associated coiled-coil containing protein kinase (RhoA/ROCK) signaling-related proteins in NCM460 cells treated with DEX and/or U46619 (RhoA/ROCK agonist) and/or DSS were detected by the respective enzyme-linked immunosorbent assay (ELISA) kits and Western blot analysis. The permeability of NCM460 monolayers was examined with transepithelial electrical resistance (TEER) assay.

**Results:** DEX had no effect on NCM460 cell viability. However, DEX improved the viability and barrier damage and suppressed the apoptosis and inflammation of DSS-induced NCM460 cells. Correspondingly, the expression of inflammation-related proteins was reduced and the expression of tight junction proteins was increased in DSS-induced NCM460 cells after treatment with DEX. In addition, RhoA/ROCK signaling was activated in NCM460 cells induced by DSS, which was suppressed by DEX. The protective effects of DEX on DSS-induced NCM460 cells were reversed by U46619.

**Conclusion:** DEX improved viability and barrier damage while suppressed apoptosis and inflammation in DSS-induced NCM460 cells by inhibiting RhoA/ROCK signaling pathway.

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<https://doi.org/10.15586/aei.v50i3.569>

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## Introduction

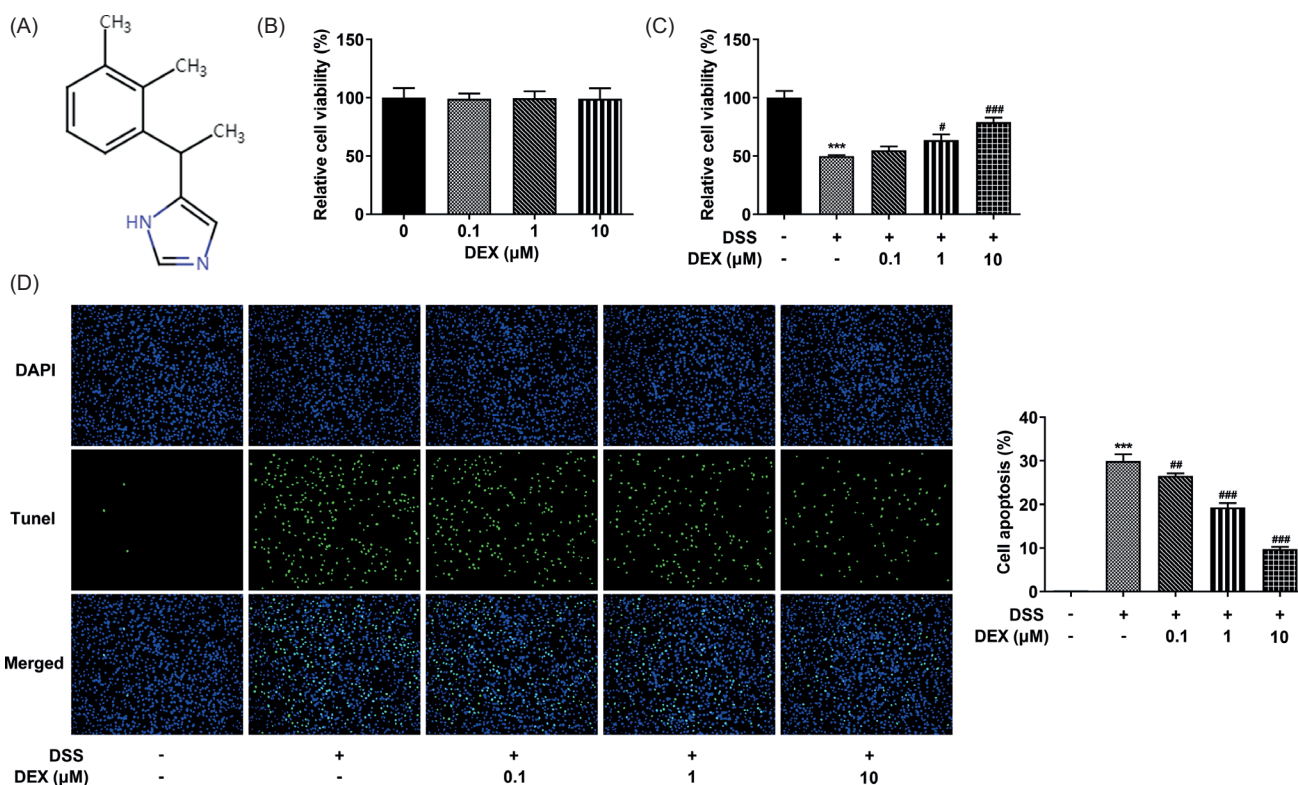
Ulcerative colitis (UC) is a frequent inflammatory bowel disease that results in inflammatory response of the colon. Disruption in intestinal epithelial barrier and continued production of pro-inflammatory cytokines play a key role in the course of ulcerative colitis.<sup>1</sup> The prevalence and morbidity of ulcerative colitis is highest in western developed countries.<sup>2</sup> Meanwhile, the morbidity rate of ulcerative colitis has also elevated among Asians and Latin Americans.<sup>3</sup> At present, corticosteroid drugs or immunosuppressants are primarily used for symptomatic treatment of inflammatory reactions. Although there are certain curative effects, long-term toxicity and adverse reactions are significant. The increased risk of cancers in patients with ulcerative colitis, which is easy to recur and has a prolonged disease course of 10-20 years, is as high as 28%.<sup>4</sup>

Dexmedetomidine (DEX) is a highly selective  $\alpha$ 2-adrenergic receptor agonist possessing anti-anxiety, sedative, sympathetic and analgesic effects, and widely used in intensive care units as an adjunct to sedation and anesthesia (Figure 1A).<sup>5</sup> DEX preconditioning effectively prevents intestinal ischemia-reperfusion (I/R) injury and inhibits TLR4/MyD88/nuclear factor  $\kappa$ B (NF- $\kappa$ B) signaling. Also, DEX could reduce the *in vivo* and *in vitro* production of inflammatory cytokines.<sup>6</sup> In addition, DEX alleviates intestinal

epithelial hyperpermeability caused by burns by reducing inflammation and enhancing the expression and distribution of tight junction proteins zonula occluden-1 (ZO-1) and occludin.<sup>7</sup> In addition, DEX has a protective effect on cecum ligation and puncture (CLP)-induced intestinal injury, which may be related to the inhibition of inflammation by regulating TLR4 pathway.<sup>8</sup> However, the function of DEX in ulcerative colitis has not been determined.

Ras homolog gene family, member A/Rho-associated coiled-coil containing protein kinase (RhoA/ROCK) pathway is closely related to a variety of cell functions, such as generation of reactive oxygen species (ROS), cell migration and differentiation etc.<sup>9</sup> ROCK belongs to a 160-kD threonine/serine kinase, primarily containing ROCK1 and ROCK2. ROCK1 and ROCK2 are key effectors of RhoA and regulate a variety of physiological functions through phosphorylation of downstream targets.<sup>10-12</sup> In the downstream of ROCK, the activation of NF- $\kappa$ B, a pivotal driving factor in the aetiology of inflammatory bowel disease (IBD), is closely related to colon and intestinal inflammation.<sup>13-15</sup> DEX may reduce myocardial apoptosis and improve cardiac function by inhibiting RhoA/ROCK signaling pathway.<sup>16</sup>

Therefore, this paper is aimed to understand the definite role of DEX in dextran sulfate sodium (DSS)-triggered inflammation and barrier damage in NCM460 cells through RhoA/ROCK signaling pathway.



**Figure 1** DEX increases viability and inhibits apoptosis of DSS-induced NCM460 cells. (A) Chemical structural formula of DEX. (B) Influence of DEX on the viability of NCM460 cells was appraised by CCK-8 assay. (C) Viability of DSS-induced NCM460 cells treated with DEX was detected by CCK-8 assay. (D) The apoptosis of DSS-induced NCM460 cells treated with DEX was determined by TUNEL assay. \*\*\*P < 0.001 vs. control group; #P < 0.05, ##P < 0.01 and ###P < 0.001 vs. DSS group.

## Materials and methods

### Cell culture

The human normal colonic epithelial cell line NCM460 acquired from BeNa Culture Collection (BNCC, Beijing, China) were maintained in modified 1640 medium (HyClone, Logan, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, CA, USA) and 1% antibiotics (Beyotime, Shanghai, China) at 37°C with 5% CO<sub>2</sub>.

DSS-treated NCM460 cells were used as an *in vitro* model for colitis. DSS 2% (MP Biomedicals, Southern California, USA) was added to the culture medium and cells were cultured for 24 h till the cell density reached about 70%. Additionally, DEX (0.1-, 1- and 10-μM) was used to pre-treat NCM460 cells for 1 h.

### Cell Counting Kit-8 (CCK-8) assay

In short, NCM460 cells grown in 96-well plates (5.5 × 10<sup>3</sup> cells/well) were pretreated with DEX for 1 h and 5% CO<sub>2</sub> at 37°C. Treated or untreated cells in each well were cultivated with 10-μL CCK8 reagent for 2 h at 37°C. The absorbance was estimated at 450 nm by a microplate reader (Thermo Fisher Multiskan FC, Waltham, MA, USA).

### TUNEL assay

After treated with DEX for 1 h and induced by DSS for 24 h, treated NCM460 cells were observed using a fluorescence microscope. This was done after 4',6-diamidino-2-phenylindole (DAPI) staining using a terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) *in situ* nick-end labeling (TUNEL) detection kit (Roche, Shanghai, China).

### Enzyme linked immunosorbent assay (ELISA)

NCM460 cells were plated into 96-well plates (5.5 × 10<sup>3</sup> cells/well). In one experiment, NCM460 cells were pretreated with DEX (0.1-, 1- and 10-μM) for 1 h and then induced by DSS for another 24 h. In another experiment 1-nM U46619 (RhoA/ROCK agonist) and 10-μM DEX were used to pretreat NCM460 cells for 1 h and then these cells were induced by DSS for another 24 h. Finally, the cell culture media were harvested and tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 levels were respectively tested using TNF-α ELISA kit (PT518), IL-1β ELISA kit (PI305) and IL-6 ELISA kit (PI330) (all from Beyotime).

### Western blot analysis

With the adoption of radioimmunoprecipitation assay (RIPA) lysis buffer (Cell Signaling Technologies, Beverly, USA), treated NCM460 cells were subjected to protein isolation at 4°C for 20 min, followed by centrifugation (10,000 × g) for 10 min at 4°C. The bicinchoninic acid (BCA) assay measured protein concentration in supernatants.

Subsequently, 30-μg total protein from each group was detached by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. After impeded with 5% non-fat milk at room temperature for 1 h, the membranes were incubated at 4°C overnight with the following specific antibodies against cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), ZO-1, occludin, claudin-1, RhoA, ROCK1, ROCK2 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Next day, the membranes were washed thrice and incubated with horseradish peroxidase (HRP)-conjugated goat-anti-rabbit secondary antibody at room temperature for 1 h. Subsequently, the membranes were visualised using a hemiluminescence kit (Millipore, Billerica, MA, USA) and Image-Pro Plus software (v6; Media Cybernetics Inc., Rockville, MD, USA) was used to analyze grey values.

### Determination of transepithelial electrical resistance (TEER)

TEER assay was used to assess the permeability of NCM460 monolayers.<sup>17</sup> NCM460 cells inoculated in 24-well transwell filters were allowed to grow until they formed a tight layer in an incubator at 37°C, 5% CO<sub>2</sub> and saturated humidity. After indicated treatment, the TEER value was estimated by an epithelial voltohmmeter (World Precision Instruments, Sarasota, FL, USA).

### Statistical analysis

Each experiment was independently performed in triplicate, and all data were displayed as mean ± standard deviation (SD). Multiple comparisons were evaluated by one-way analysis of variance followed by Tukey's test. Differences between two groups were determined by *t*-test; *P* < 0.05 implied statistically significant.

## Results

### DEX increases viability and inhibits apoptosis of DSS-induced NCM460 cells

It was observed that the viability of NCM460 cells was not changed after administrated with 0.1-, 1- and 10-μM DEX (Figure 1B). The viability was inhibited and apoptosis was promoted in NCM460 cells after DSS induction; this all was reversed by DEX (Figures 1C and D).

### DEX suppresses inflammatory response in DSS-induced NCM460 cells

The levels of TNF-α, IL-1β and IL-6, which were deemed as inflammatory factors, and the expressions of inflammation-related factors (COX-2 and iNOS) were significantly increased in DSS-induced NCM460 cells (Figures 2A and B). However, DEX hampered inflammation dependent on increasing concentrations.

### DEX inhibits barrier damage in DSS-induced NCM460 cells

As exhibited in Figure 3A, DSS reduced the level of TEER while DEX improved the reduced TEER. Besides, ZO-1, occludin and claudin-1 protein levels were downregulated in NCM460 cells exposed to DSS. This DSS-induced alteration of tight junction protein expression was reversed by DEX (Figures 3B and C).

### DEX inhibits the RhoA/ROCK signaling pathway in DSS-induced NCM460 cells

In order to probe the association of DEX with RhoA/ROCK pathway activation stimulated by DSS, Western blot analysis was used to test the protein levels of RhoA, ROCK1 and ROCK2. It turned out that expressions of RhoA, ROCK1 and ROCK2 were elevated in the DSS group. However, these effects were significantly inhibited by DEX (Figure 4).

### U46619 weakens the inhibiting effect of DEX on inflammation in DSS-induced NCM460 cells

Regarding the protective effects of different concentrations of DEX observed in previous studies, 10- $\mu$ M DEX was selected for subsequent experiment. U46619 weakened the protective effects of DEX on inflammation, as reflected by the upregulated levels of inflammatory factors (TNF- $\alpha$ , IL-1 $\beta$  and IL-6; Figure 5A) and increased expressions of inflammation-related proteins (COX-2 and iNOS; Figure 5B).

### U46619 weakens the inhibiting effect of DEX on barrier damage in DSS-induced NCM460 cells

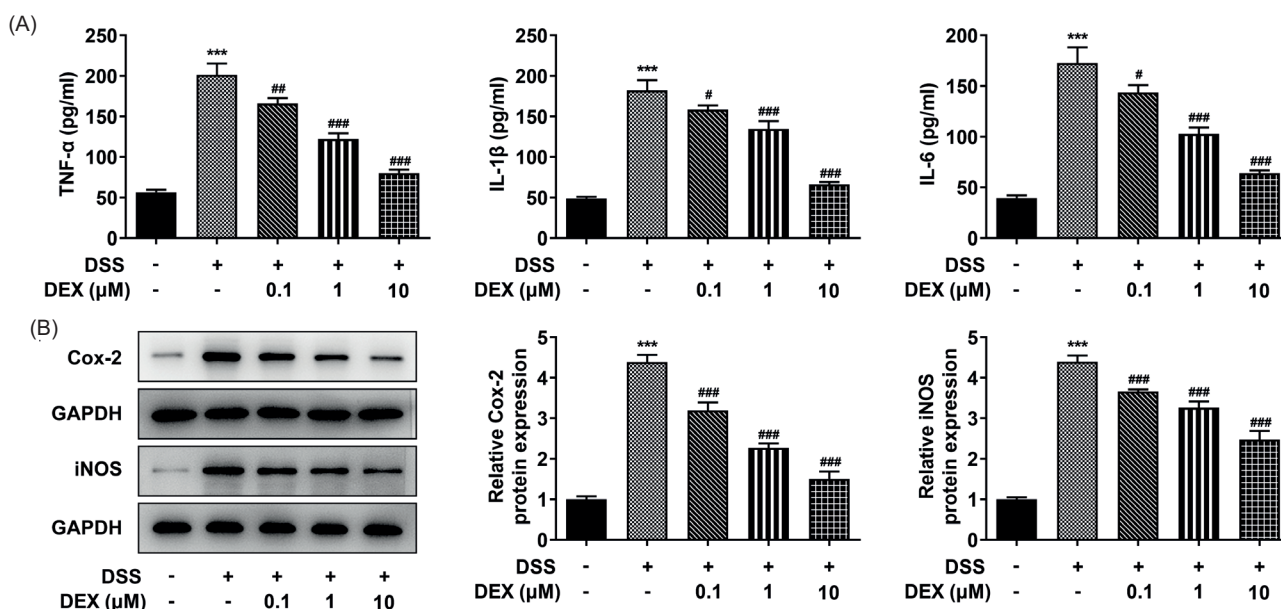
U46619 decreased the level of TEER in DSS-induced NCM460 cells treated with DEX (Figure 6A). Correspondingly, expressions of ZO-1, occluding and claudin-1 were downregulated after U46619 treatment in DSS-induced NCM460 cells (Figure 6B).

## Discussion

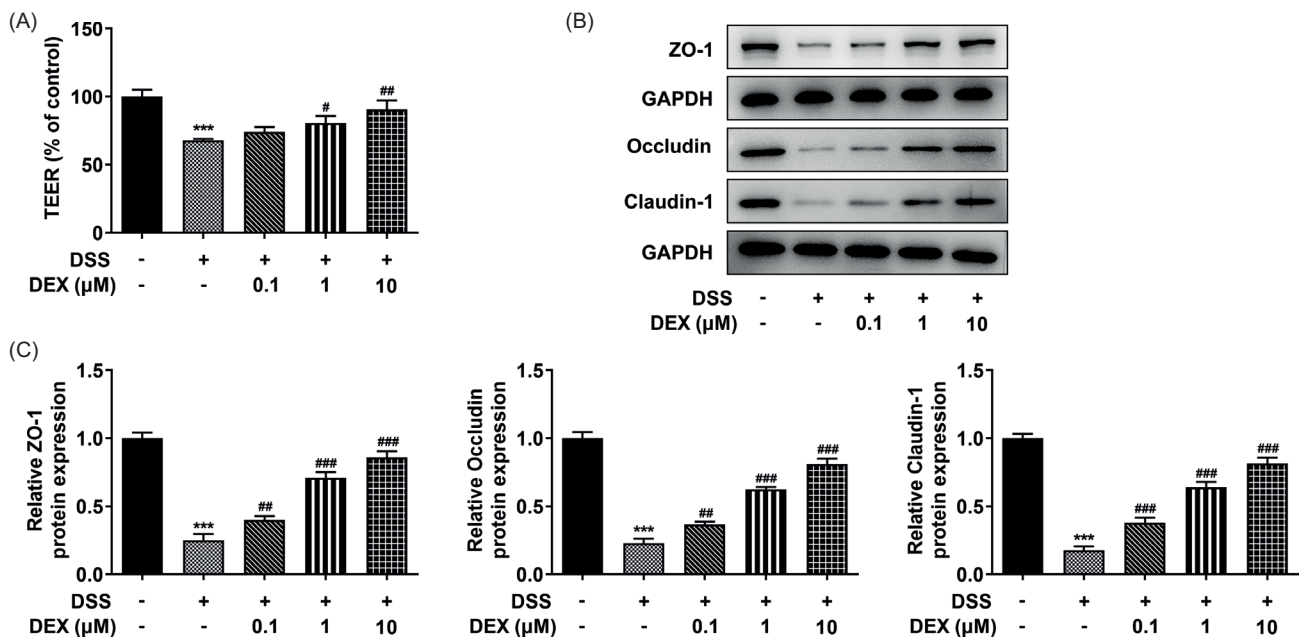
Presently, treatments for ulcerative colitis continue to develop, but available therapies are limited to symptomatic relief and non-specific immunomodulators.<sup>18</sup> Therefore, the treatment of ulcerative colitis remains an area of significant medical requirement.

It is determined that high levels of pro-inflammatory mediators such as cytokines (TNF- $\alpha$ , IL-1 $\beta$  and IL-6) is a dominant feature of ulcerative colitis which leads to chronic inflammatory ulcers and in turn leukocyte infiltration of colonic mucosal injury.<sup>19</sup> TNF- $\alpha$  is deemed as a driving factor for the destruction of epithelial barrier, inducing apoptosis of epithelial cells. IL-6 is related to the recruitment and activation of neutrophils, exacerbating the inflammatory state of inflammatory enteritis. Excessive production of IL-1 $\beta$  and IL-6 may lead to increased intestinal inflammation and intestinal motility disorders.<sup>18</sup>

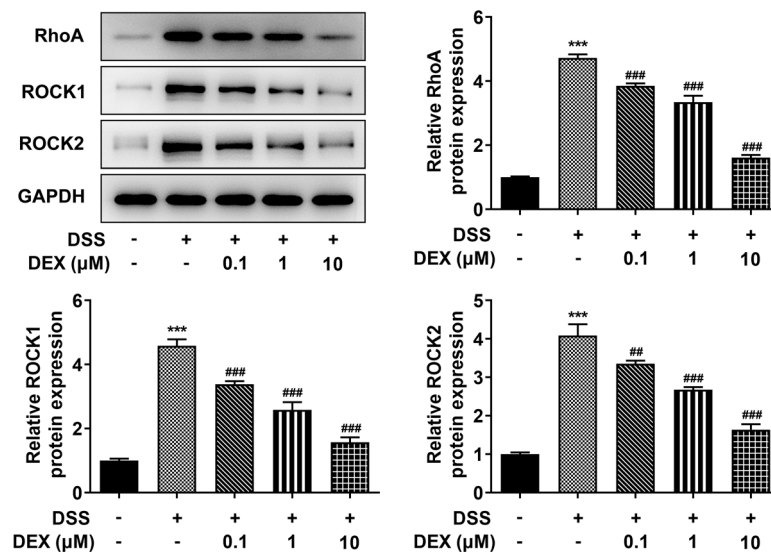
COX-2 is an important target gene product of hypoxia regulatory factors and is engaged in the regulation of apoptosis, angiogenesis, inflammation and tumorigenesis.<sup>20,21</sup> *In vivo* prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), nitric oxide (NO) and IL-17



**Figure 2** DEX suppresses inflammatory response in DSS-induced NCM460 cells. (A) Levels of inflammatory factors in DSS-induced NCM460 cells treated with DEX were determined by their commercial kits. (B) Western blot analysis determined the influence of DEX on the expression of inflammation-related factors in DSS-treated NCM460 cells. \*\*\*P < 0.001 vs. control group; #P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001 vs. DSS group.



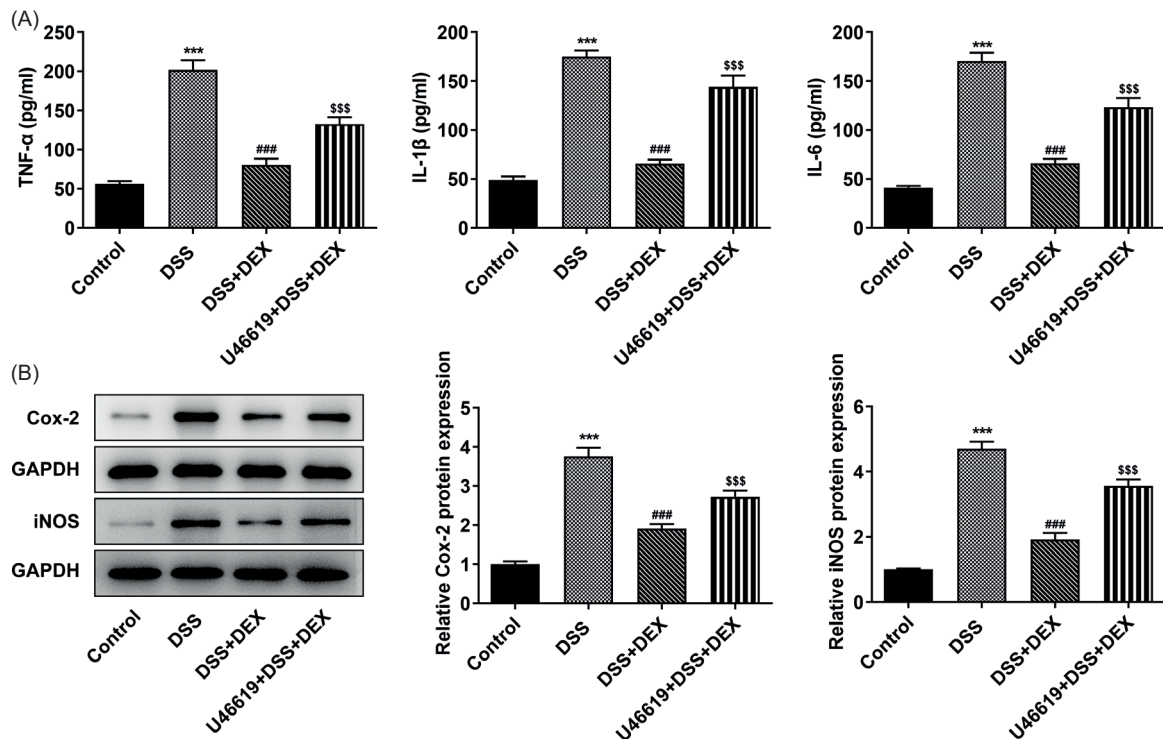
**Figure 3** DEX inhibits barrier damage in DSS-induced NCM460 cells. (A) The permeability of DSS-induced NCM460 monolayers treated with DEX was detected by TEER measurement. (B) and (C) Expression of tight junction proteins in DSS-induced NCM460 cells treated with DEX was detected by Western blot analysis. \*\*\* $P < 0.001$  vs. control group. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. DSS group.



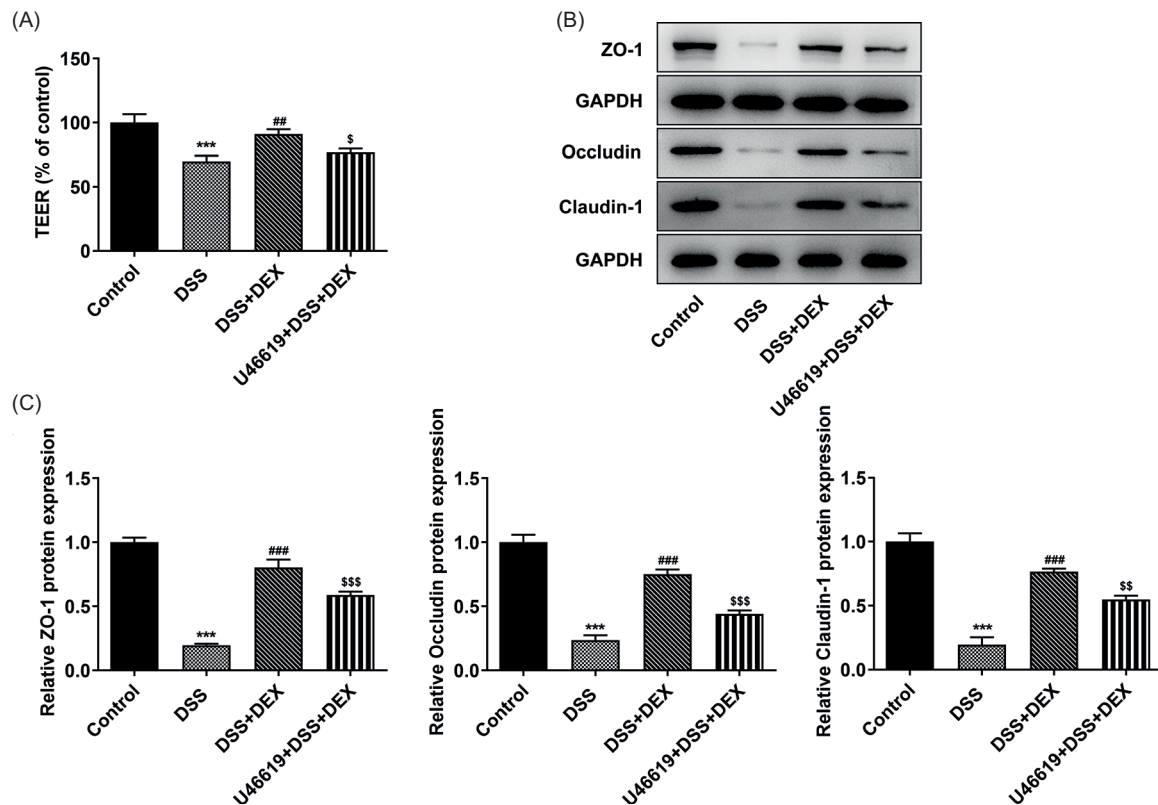
**Figure 4** DEX inhibits the RhoA/Rock signaling pathway in DSS-induced NCM460 cells. Expression of RhoA/ROCK signaling pathway-related proteins in DSS-induced NCM460 cells treated with DEX was determined by Western blot analysis. \*\*\* $P < 0.001$  vs. control group. \*\* $P < 0.01$  and \*\*\* $P < 0.001$  vs. DSS group.

demonstrated strong pro-inflammatory response. iNOS is a protein-independent enzyme primarily expressed in inflammatory cells and is an important factor in inducing NO production.<sup>22,23</sup> COX-2 and iNOS are key upstream enzymes for the synthesis of PGE2 and NO, and their abundance expression can directly limit the production of downstream PGE2 and NO.<sup>24</sup> In this study, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 levels and COX-2 and iNOS expressions were enhanced in DSS-exposed NCM460 cells which were lessened after DEX treatment.

Intestinal mucosal barrier function plays an important role in the development of ulcerative colitis.<sup>25,26</sup> The intestinal mucosal barrier is the first barrier against harsh environment and is primarily formed by tight junctions (TJs) of epithelial cells. Tight junctions consist of transmembrane proteins (occludins and claudins) and helper protein (ZO) to prevent the spread of pathogens and harmful antigens within the epithelium.<sup>27</sup> Zonula occludens, occludins and claudins can seal gap between adjacent intestinal epithelial



**Figure 5** U46619 weakens the inhibiting effect of DEX on inflammation in DSS-induced NCM460 cells. (A) Levels of inflammatory factors in DSS-induced NCM460 cells treated with DEX and U46619 were determined by their commercial kits. (B) Western blot analysis tested the impact of DEX and U46619 on the expression of inflammation-related factors in DSS-treated NCM460 cells. Expression of inflammation-related proteins in DSS-induced NCM460 cells treated with DEX and U46619 was detected by Western blot analysis. \*\*\*P < 0.001 vs. control group; ###P < 0.001 vs. DSS group; <sup>SSS</sup>P < 0.001 vs. DSS+DEX group.



**Figure 6** U46619 weakens the inhibiting effect of DEX on barrier damage in DSS-induced NCM460 cells. (A) The permeability of DSS-induced NCM460 monolayers treated with DEX and U46619 was detected by TEER measurement. (B) and (C) Expression of tight junction proteins in DSS-induced NCM460 cells treated with DEX and U46619 was detected by Western blot analysis. \*\*\*P < 0.001 vs. control group. ##P < 0.01 and ###P < 0.001 vs. DSS group. <sup>S</sup>P < 0.05, <sup>SS</sup>P < 0.01 and <sup>SSS</sup>P < 0.001 vs. DSS+DEX group.

cells and retain antigens and microorganisms in the intestinal lumen which occupies a significant position for maintaining intestinal permeability, tissue differentiation and internal environment stability.<sup>28</sup> Therefore, it is important to regulate tight junctions to maintain integrity of the ulcerative colitis epithelial barrier. Here, ZO-1, occludin and claudin-1 expressions were declined apparently, accompanied by the decreased TEER level. Nonetheless, DEX could reverse the above results.

ROCK, chief effector in the downstream of RhoA, has been demonstrated to regulate biological pathways, affecting smooth muscle tension levels and various physiological characteristics related to actin cytoskeletal changes.<sup>29,30</sup> Recent reports have documented that the RhoA/ROCK signaling pathway is implicated in the activation of immune system and brings about the release of pro-inflammatory factors.<sup>11</sup> Brusatol-enriched *Brucea javanica* oil (BE-BJO) inhibited NF- $\kappa$ B and RhoA/ROCK signaling pathway by suppressing colonic inflammation and enhancing intestinal epithelial barrier function to ameliorate DSS ulcerative colitis.<sup>31</sup> Oxymatrine could relieve DSS-induced acute intestinal inflammation by blocking RhoA/ROCK signaling pathway to protect the integrity of epithelial barrier, alleviate inflammation and oxidative stress, and maintain balance between Th17 and Treg cells.<sup>32</sup> Our experimental results established that the RhoA/ROCK signaling pathway was activated in NCM460 cells after DSS induction, which was suppressed by DEX. In addition, U46619, an agonist of RhoA/ROCK signaling pathway, could weaken the effect of DEX on DSS-induced NCM460 cells to promote inflammation and reduce TEER level.

## Conclusion

Our results indicated that DEX could protect against inflammation and barrier damage in DSS-induced NCM460 cells by inhibiting the RhoA/ROCK signaling pathway. These findings confirmed a therapeutic potential of DEX in ulcerative colitis. Are there other signaling pathways involved in DEX effect on DSS-treated NCM460 cells is required to be explored in the future research.

## Competing interests

The authors declared that they have no competing interests.

## Availability of data and material

The experimental data will be available on request.

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